EFFECT OF AGROCHEMICALS ON RHIZOTROPHIC MICROFLORA AND LEGUME-*RHIZOBIUM* SYMBIOSIS

SUMMARY
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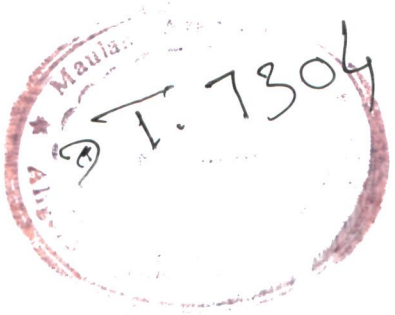

Summary

In high input agronomic practices, pesticides are frequently used to prevent the organisms causing detrimental effect on crop plants and consequently to increase the crop productivity. After application, a large portion of pesticides persist in soils and pose a major threat to both microbial diversity and crop productivity. A well recognized practice for maintaining soil fertility has however, been the cultivation of legume crops, which add substantial amount of N to the soil by forming effective symbiosis with N_2 fixing organisms. Besides N_2 fixers, other plant growth promoting rhizobacteria (e.g. P solubilizers) are also used in agriculture production systems. And hence, microbial inoculants are commonly applied to seeds to ensure effective crop yields. The inoculant is, however, often used in conjunction with agrochemicals, which indeed contain essential nutrients to facilitate plant growth besides containing toxic substances. Of these chemicals, pesticides though applied to restrict the harmful effect of insect pests both in conventional and derelict soils may also be potentially hazardous and lead to losses in crop productivity.

Due to inadequate and conflicting reports on the toxicity of pesticides on plant growth promoting rhizobacteria and legume-*Rhizobium* symbiosis and the possibility of damage to the legumes due to the application of pesticides into the soils, it was desirable to explore the diversity of plant growth promoting rhizobacteria in terms of their functional variation in the Aligarh district of Western Uttar Pradesh, India. Subsequently, the toxicity of certain pesticides to the functional properties of selected PGPR and the effect of pesticides and pesticide tolerant PGPR strains on popularly grown legumes in the region was investigated. The present investigation was therefore, designed with specific objectives:-

1. To assess soil microbial diversity in different rhizospheres of popularly grown crops grown in this area.
2. To isolate nitrogen fixing bacteria from the nodules of legumes, chickpea, pea, greengram and lentil and phosphate solubilizing bacteria from mustard rhizosphere.
3. To evaluate the tolerance of rhizobacteria (nitrogen fixers and phosphate solubilizing bacteria) and growth pattern against selective herbicides (quizalafop-p-ethyl, clodinafop, metribuzin and glyphosate), insecticides (fipronil, pyriproxyfen, imidacloprid and thiamethoxam) and fungicides (tebuconazole, hexaconazole, metalaxyl and kitazin).

4. To assay both qualitatively and quantitatively the production of plant growth promoting substances by PGPR strains.

5. To assess the *in vitro* biotoxicity of pesticides to plant growth promoting traits of both nitrogen fixers and phosphate solubilizers and to identify pesticide tolerant strains for their PGP activities under pesticide stressed condition.

6. To evaluate the phytotoxic effects of recommended and higher doses of selective pesticides including herbicides (quizalafop-p-ethyl and clodinafop), insecticides (fipronil and pyriproxyfen) and a fungicide (tebuconazole) on the biological and chemical properties of chickpea, greengram, lentil and pea preferably grown in the vicinity of Aligarh.

7. To evaluate the effect of the pesticide tolerant plant growth promoting rhizobacteria on the performance of chickpea, greengram, lentil and pea plants grown in pesticide stressed soils.

The rhizospheric soils of chickpea, greengram, lentil, pea and mustard grown at the experimental fields of Faculty of Agricultural Sciences, A.M.U., Aligarh, were used to assess the microbial diversity. The bacterial populations in the rhizosphere of chickpea, greengram, lentil and pea were 3.21 x 10^7, 2.86 x 10^7, 3.53 x 10^7 and 3.11 x 10^7 CFU/g soil, respectively. The rhizospheric soils of mustard showed a substantial increase of 36, 52, 23 and 29% in bacterial populations compared to those recovered from chickpea, greengram, lentil, and pea, respectively. The fungal populations in all the rhizospheric soils ranged from 1.2 x 10^5 (lentil) to 2.1 x 10^5 (greengram) CFU/g soil. In general, the populations of phosphate solubilizing bacteria (PSB) were more than the phosphate solubilizing fungi (PSF) in all soil samples. Furthermore, a total of 50 N_2-fixing strains belonging to the genera *Mesorhizobium*, *Bradyrhizobium* and *Rhizobium* were isolated from the nodules of chickpea, greengram, lentil and pea crops using yeast extract mannitol agar plates while 50 strains of PSB were isolated from the rhizospheric soils of mustard. The isolated bacterial strains were characterized morphologically and biochemically. Among PSB, four isolates (PS1, PS2, PS9 and PS19) showing highest degree of TCP solubilization, were selected for further molecular characterization. These isolates were shown to belong to the genera *Pseudomonas aeruginosa* [PS1 (Gene Bank accession number FJ705886)], *Enterobacter asburiae* [PS2 (Gene Bank accession number FJ705887)], *Pseudomonas putida* [PS9 (Gene Bank accession number FJ705888) and *Klebsiella sp.* [PS19 (Gene Bank accession number FJ705889) by partial sequencing analysis of their respective 16s rDNA genes. Among the bacterial strains, 22% of *Mesorhizobium* spp. (chickpea), 18% of *Bradyrhizobium* spp.

(greengram), 14% of *Rhizobium* spp. (pea), 16% of *Rhizobium* spp. (lentil) and 36% of PSB were selected for assaying further the plant growth promoting activities. The mesorhizobial strains. rhizobial strains (pea), bradyrhizobial strains, rhizobial strains (lentil), phosphate solubilizing bacterial strains were grouped into four, three, three, four and four PGP groups, respectively.

The PGPR strains showing greatest plant growth promoting activities *in vitro* were selected to evaluate the toxic effects of varying concentrations of herbicides (quizalafop-p-ethyl, clodinafop, glyphosate, metribuzin), insecticides (fipronil, pyriproxyfen, imidacloprid, thiamethoxam), fungicides (tebuconazole, hexaconazole, metalaxyl and kitazin) using agar plate dilution method in order to identify the pesticide tolerant PGPR strains. Strains MRC4, MRP1, MRM6 and MRL3 showed the highest tolerance to most of the pesticides among *Mesorhizobium* spp., *Rhizobium* spp. (pea), *Bradyrhizobium* spp. and *Rhizobium* spp. (lentil), respectively. In contrast, of the 18 PSB, *Pseudomonas aeruginosa* PS1, *Enterobacter* asburiae PS2, *Pseudomonas putida* PS9 and *Klebsiella* sp. PS19, tolerated most of the tested pesticides. Of all the PGPR strains, *Pseudomonas aeruginosa* PS1 was the most tolerant bacterium which had a maximum resistance level (MRL) values for all the herbicides, insecticides and fungicides. Furthermore, growth pattern of the tolerant strains, *Mesorhizobium* strain MRC4, *Rhizobium* strain MRP1, *Bradyrhizobium* strain MRM6, *Rhizobium* isolate MRL3 and *Pseudomonas aeruginosa* PS1 grown in minimal media supplemented with different concentrations of 12 pesticides at different incubation periods showed a substantial variation.

The production of IAA by the selected bacterial strains namely, *Mesorhizobium* spp. (N=11), *Rhizobium* spp. (pea, N=7), *Bradyrhizobium* spp. (N=9), *Rhizobium* spp. (lentil, N=8) and PSB (N=18) was assayed in LB broth supplemented with a fixed concentration (100 µg/ml) of tryptophan. A wide range of variation in the secreted amount of IAA was observed among rhizobial isolates. Generally, the amount of IAA released by PGPR strains varied between 14 (MRC10) to 44 µg /ml (MRC4) for mesorhizobial strains, 17 (MRP4) to 32 µg /ml (MRP1) for pea specific *Rhizobium* isolates, 15 (MRM7) to 38 µg /ml (MRM6) for bradyrhizobial strains and 15 (MRL2, MRL7) to 37 µg/ml (MRL3) for *Rhizobium* strains isolated from lentil nodules. Of phosphate solubilizing bacteria (N=18), *Klebsiella* sp. PS19 was the most efficient strains and produced a highest amount of IAA (42 µg/ml) which was followed by *Pseudomonas aeruginosa* PS1 (39 µg/ml), *Pseudomonas putida* PS9 (34 µg/ml), *Enterobacter asburiae* PS2 (32 µg/ml) under normal growth conditions. Furthermore, a total of 36% of the *Mesorhizobium* strains

produced siderophore on CAS agar plates five days after incubation and the halo size for siderophores varied between 9 (MRC10) to 12 mm (MRC4). Further, the ethyl acetate extraction from culture supernatant of *Mesorhizobium* strain MRC1 yielded 30 and 17 µg/ml salicylate (SA) and 2,3-dihydroxy benzoic acid (DHBA), strain MRC4 produced 35 and 19 µg/ml of SA and DHBA, strain MRC7 yielded 25 and 18 µg/ml SA and DHBA, and strain MRC10 yielded 21 and 17 µg/ml SA and DHBA, respectively. Among the *Rhizobium* species isolated from pea nodules, only three (43%) strains were positive for siderophore activity where strain MRP1, MRP4 and MRP7 demonstrated 11, 10 and 11 mm orange colored zone on CAS agar plates. Further, these strains produced 32 and 22 (strain MRP1), 29 and 18 (MRP4) and 25 and 14 (MRP7) µg/ml SA and DHBA, respectively. Strains MRM3, MRM6 and MRM8 of *Bradyrhizobium* species showed 10, 13 and 11 mm colored zone, respectively, on CAS agar plates and produced 30 and 15 (strain MRM3), 32 and 18 (MRM6) and 28 and 16 (MRM8) µg/ml SA and DHBA, respectively. Among the *Rhizobium* species isolated from lentil nodules, 50% of the rhizobial isolates showed a positive reaction to siderophore both on CAS agar plates and in liquid culture medium. The siderophore zone size produced by such strains ranged between 10 (strain MRL1, MRL3, MRL7) to 12 mm (MRL6) and yielded 26 and 18 (MRL1), 29 and 21 (MRL3), 27 and 17 (MRL6) and 25 and 15 µg/ml SA and DHBA, respectively. Among the siderophore producing phosphate solubilizers (55%), *Pseudomonas aeruginosa* PS1, *Enterobacter asburiae* PS2, *Pseudomonas putida* PS9 and *Klebsiella* sp. PS19 had 15, 13, 14 and 14 mm colored zone, respectively on CAS plates. In liquid culture medium, *Pseudomonas aeruginosa* PS1 showed 41 and 21 µg/ml of SA and DHBA production. *Enterobacter asburiae* PS2 produced 24 and 9, *Pseudomonas putida* PS9 produced 41 and 17 and *Klebsiella* sp. PS19 produced 47 and 10 µg/ml of SA and DHBA, respectively.

The exo-polysaccharides (EPS) synthesized by the pesticide tolerant bacterial strains were determined after 120 h of incubation. Among the bacterial strains, a total of 36, 43, 33, 38 and 100% of mesorhizobia, rhizobia (pea), bradyrhizobia, rhizobia (lentil) and PSB respectively, secreted EPS in liquid culture medium. Particularly, *Mesorhizobium* strain MRC4, *Rhizobium* strain MRP1, *Bradyrhizobium* strain MRM6, *Rhizobium* strain MRL3, *Pseudomonas aeruginosa* PS1, *Enterobacter asburiae* PS2, *Pseudomonas putida* PS9 and *Klebsiella* sp. PS19 produced 21, 20, 21, 18, 18, 16, 17 and 18 µg/ml EPS, respectively. The plant growth promoting rhizobacteria were further evaluated for their phosphate solubilizing potential, both on solid and in liquid

Pikovskaya medium. A total of 34% rhizobacterial strains showed the phosphate solubilizing activity and formed a clear halo around their growth. Generally, the size of phosphate solubilizing zone on solid Pikovskaya ranged from 4 (*Bacillus* sp. PS4) to 14 mm (*Klebsiella* sp. PS19). The solubilization index (SI) ranged between 0.5 (*Bacillus* PS 4) to 2.5 (*Klebsiella* PS 19). Similarly, a considerable amount of tri-calcium phosphate (TCP) was solubilized in liquid culture by *Pseudomonas aeruginosa* PS1 (345 µg/ml), *Enterobacter asburiae* PS2 (258 µg/ml), *Pseudomonas putida* PS9 (298 µg/ml) and *Klebsiella* sp. PS19 (294 µg/ml). The solubilization of TCP was accompanied by decrease in pH of the medium. In addition, all growth promoting rhizobacterial strains of *Bradyrhizobium*, *Rhizobium* (lentil) and phosphate solubilizers showed a positive reaction for ammonia. In contrast, only 91% of *Mesorhizobium* and 86% of *Rhizobium* (pea) were positive for ammonia Furthermore, a total of 63% *Mesorhizobium*, 100% *Rhizobium* (pea), 33% *Bradyrhizobium*, 75% *Rhizobium* (lentil) and 50% phosphate solubilizing strains were found to be positive for HCN production.

A total of eight pesticide tolerant rhizobacterial strains including *Mesorhizobium* strain MRC4, *Rhizobium* strain MRP1 (pea), *Bradyrhizobium* strain MRM6, *Rhizobium* strain MRL3 (lentil), *Pseudomonas aeruginosa* strain PS1, *Enterobacter asburiae* strain PS2, *Pseudomonas putida* strain PS9 and *Klebsiella sp.* strain PS19 were evaluated further for plant growth promoting activities in their respective medium supplemented with varying concentrations of selected pesticides. In this study, the effect of three concentrations (recommended dose – X, double of recommended dose – 2X and three times more of recommended dose – 3X) of herbicides (quizalafop-p-ethyl, clodinafop, metribuzin and glyphosate), insecticides (fipronil, pyriproxyfen, imidacloprid and thiamethoxam) and fungicides (tebuconazole, hexaconazole, metalaxyl and kitazin) on IAA synthesized by rhizobacterial strains was determined in LB broth treated with 100 µg/ml of tryptophan. The rhizobacterial strains in general through produced a considerable amount of IAA but IAA decreased progressively with increase in concentrations of herbicides, insecticides and fungicides. In case of herbicides, metribuzin had the least toxic effect on IAA synthesis while quizalafop-p-ethyl had a profound adverse effect on IAA production by mesorhizobial strain MRC4. Of all the herbicides, metribuzin reduced the IAA production by 16% while quizalafop-p-ethyl by 75% at 3X concentration, over untreated control. Among insecticides, pyriproxyfen affected IAA synthesis most severely and decreased it by 62% while fungicide tebuconazole decreased it by 75% at 3X, relative to control. Among herbicides,

quizalafop-p-ethyl displayed most toxic effect on IAA produced by *Rhizobium* strain MRP1 and decreased it by 44% at 3X over control. Among insecticides, fipronil showed highest toxicity and decreased IAA production by 35% at 3X in comparison to control while tebuconazole, among fungicides, demonstrated the greatest toxicity on IAA and declined it by 50% at 3X, compared to control. *Bradyrhizobium* strain MRM6 when grown in LB medium amended with normal rates of quizalafop-p-ethyl, clodinafop, metribuzin and glyphosate produced maximum amount of 7, 17, 30 and 28 µg/ml IAA which significantly declined by 8%, 18%, 32% and 40% respectively, at 3X of each herbicide over control. Among three concentrations of each herbicide, the 3X of quizalafop-p-ethyl was most toxic and reduced the production of IAA by 57% compared to those observed for normal rate of the same herbicide. Of the three concentrations of each insecticide, the 3X of both fipronil and pyriproxyfen showed the most toxic effect and reduced the IAA biosynthesis by 56% relative to those observed for recommended rates of the same insecticides. Like the effect of 3X of both herbicides and insecticides, the 3X of fungicides in general, also had a greatest toxic effect on IAA synthesis by *Bradyrhizobium* MRM6; the maximum being observed for 3X of tebuconazole which reduced IAA by 89% over untreated control. In the presence of different concentrations of herbicides insecticides and fungicides, IAA production by *Rhizobium* strain MRL3 decreased progressively as the concentration of each pesticide was increased gradually from recommended to the highest tested dose.

Phosphate solubilizing bacteria namely, *Pseudomonas aeruginosa* PS1, *Enterobacter asburiae* PS2, *Pseudomonas putida* PS9 and *Klebsiella* sp. PS19 tolerant to herbicides, insecticides and fungicides were also tested for IAA synthesis under pesticide stressed environment. Even though, these bacterial strains produced IAA but in general, the synthesis of IAA by the P-solubilizers decreased consistently with increasing concentrations of herbicides, insecticides and fungicides. However, production of IAA by the four selected pesticide tolerant and P solubilizing strains (*Pseudomonas aeruginosa* PS1, *Enterobacter asburiae* PS2, *Pseudomonas putida* PS9 and *Klebsiella* sp. PS19) under pesticides stress conditions did not differ significantly. The biotoxicity of quizalafop-p-ethyl among herbicides, pyriproxyfen within insecticides and tebuconazole in fungicides group was most prominent over bacterial IAA biosynthesis. Quizalafop-p-ethyl decreased the synthesis of IAA by 90, 91, 88 and 84%, pyriproxyfen by 85, 72, 80 and 79% and tebuconazole by 92, 94, 95 and 93% at 3X by *Pseudomonas aeruginosa* PS1, *Enterobacter asburiae* PS2, *Pseudomonas putida* PS9 and

Klebsiella sp. PS19, respectively. Though the order of biotoxicity of pesticides on bacterial IAA biosynthesis was not uniform, however, at three times of recommended dose, the order of toxicity of pesticides on the IAA synthesis by *Pseudomonas aeruginosa* PS1, the most tolerant PSB strain to all herbicides, insecticides and fungicides was: quizalafop-p-ethyl > clodinafop > glyphosate > metribuzin; pyriproxyfen > imidacloprid > fipronil > thiamethoxam and tebuconazole > hexaconazole > metalaxyl > kitazin, respectively.

Production of siderophores by the pesticide tolerant strains of PGPR was also determined both qualitatively and quantitatively in the medium supplemented with or without varying concentrations of pesticides. Generally, the tested PGPR strains showed siderophore activity on pesticides amended CAS agar plates. The size of siderophore zone produced on CAS agar plates by PGPR strains decreased with increasing concentrations of each pesticide. Furthermore, the amount of SA and DHBA, respectively, in the supernatant of *Mesorhizobium* strain MRC4 decreased consistently with increasing dose of each pesticide. Quizalafop-p-ethyl at 3X have shown maximum toxicity and decreased SA and DHBA by 46% and 48% respectively, compared to control. Among insecticides, the most prominent inhibitory effect on SA and DHBA production was recorded for pyriproxyfen which decreased SA and DHBA by 40% and 37% at 3X. Among fungicides, tebuconazole and hexaconazole affected the siderophores activity most severely. Tebuconazole reduced the production of SA and DHBA by 40 and 58% while hexaconazole by 40 and 48% respectively, at 3X over control. Additionally, the amount of SA and DHBA in the supernatant of *Rhizobium* strain MRP1 decreased consistently with increasing dosage of each pesticide. Of all the herbicides, quizalafop-p-ethyl displayed maximum toxicity and decreased SA by 57% while it reduced the DHBA by 55% at 3X, over control. The most toxic effect on SA and DHBA production was shown, among insecticides, by pyriproxyfen which decreased SA by 35% and DHBA by 46% at 3X,. Fipronil and thiamethoxam slightly reduced the siderophore activity and showed a similar pattern of SA and DHBA inhibition following strain MRP1 inoculation. Among fungicides, tebuconazole affected the siderophores production most severely and inhibited SA by 44% and DHBA by 60% at 3X, relative to control. Besides, quizalafop-p-ethyl at 3X displayed the maximum toxicity and decreased SA by 62 and 48% and DHBA by 72 and 57% for *Bradyrhizobium* strain MRM6 and *Rhizobium* strain MRL3, respectively, over respective control. Likewise, pyriproxyfen at 3X showed maximum toxicity and decreased SA by 34 and 28% and DHBA by 33 and 57% for

Bradyrhizobium strain MRM6 and *Rhizobium* strain MRL3, respectively, compared to their respective control. On the other hand, tebuconazole at 3X decreased both SA and DHBA by 44 and 52% for *Bradyrhizobium* strain MRM6 and *Rhizobium* strain MRL3, over their control.

Phosphate solubilizing bacterial strains *Pseudomonas aeruginosa* PS1, *Enterobacter asburiae* PS2, *Pseudomonas putida* PS9 and *Klebsiella* sp. PS19 tolerant to pesticides were also tested for siderophore production which decreased consistently with increasing concentrations of pesticides. Among all herbicides, quizalafop-p-ethyl at 3X displayed the maximum decrease in production of SA by 35, 68, 46 and 47% and of DHBA by 48, 78, 89 and 90% for *Pseudomonas aeruginosa* PS1, *Enterobacter asburiae* PS2, *Pseudomonas putida* PS9 and *Klebsiella* sp. PS19, respectively, over respective control. Similarly, among insecticides, pyriproxyfen at 3X showed maximum biotoxicity to SA and decreased it by 52, 47, 36 and 47% and to DHBA by 80, 83, 67 and 70% for *Pseudomonas aeruginosa* PS1, *Enterobacter asburiae* PS2, *Pseudomonas putida* PS9 and *Klebsiella* sp. PS19, respectively, compared to their respective control. Tebuconazole (fungicide) at 3X, decreased SA to highest degree by 54, 69, 58 and 52% and DHBA by 70, 77, 67 and 77% for *Pseudomonas aeruginosa* PS1, *Enterobacter asburiae* PS2, *Pseudomonas putida* PS9 and *Klebsiella* sp. PS19, respectively over control. Exo-polysaccharides (EPS) synthesized by all PGPR strains increased progressively with gradual enhancement in pesticide concentrations. For instance, glyphosate at 3X, among herbicides, increased the EPS by 23% over control. Of insecticides, imidacloprid increased EPS by 38% while fungicide hexaconazole by 33% at 3X compared to control. For *Rhizobium* strain MRP1 specific to pea, glyphosate, pyriproxyfen and tebuconazole increased EPS by 40, 30 and 25% respectively, at 3X over control. On the other hand, for *Bradyrhizobium* strain MRM6, glyphosate increased EPS by 38%, fipronil, pyriproxyfen and thiamethoxam by 23%, tebuconazole and hexaconazole by 28% at 3X compared to control. Unlike the marginal increment in EPS synthesis by *Rhizobium* strain MRL3 (lentil) in the presence of metribuzin and glyphosate, both quizalafop-p-ethyl and glyphosate at 3X increased EPS by 33% when compared with untreated control. Similarly, imidacloprid at 3X substantially increased EPS secretion by 44% compared to control. On the other hand, hexaconazole at three times of recommended rate, was found the most potential inducer of bacterial EPS secretion and increased it by 50% over control. The synthesis of EPS by the P solubilizers increased consistently with increasing concentration of each herbicides, insecticides and fungicides. The trend of EPS production by P solubilizing strains like

Pseudomonas aeruginosa PS1, *Enterobacter asburiae* PS2, *Pseudomonas putida* PS9 and *Klebsiella* sp. PS19 under pesticide stress was not uniform. In general, the effect of glyphosate (among herbicides), pyriproxyfen (insecticides) and hexaconazole (among fungicides) on bacterial EPS secretion was most obvious compared to their respective control. For example, glyphosate increased the synthesis of EPS by 38, 43, 47 and 38%, pyriproxyfen by 50, 37, 35 and 33% and hexaconazole by 56, 55, 41 and 61% at 3X by *Pseudomonas aeruginosa* PS1, *Enterobacter asburiae* PS2, *Pseudomonas putida* PS9 and *Klebsiella* sp. PS19, respectively over their respective control.

The rhizobacterial strains were further tested for HCN and ammonia production under *in vitro* conditions in the presence of three concentrations of twelve pesticides. Interestingly, the three concentrations of herbicides, insecticides and fungicides did not affect negatively HCN and ammonia synthesis each by *Mesorhizobium*, *Rhizobium* specific to pea, *Bradyrhizobium*, *Rhizobium* specific to lentil and phosphate solubilizing strains of *Pseudomonas aeruginosa* PS1, *Enterobacter asburiae* PS2, *Pseudomonas putida* PS9 and *Klebsiella* sp. PS19. The phosphate solubilizing potentials of the PGPR strains in the presence of varying concentrations of herbicides, insecticides and fungicides was also assayed both qualitatively and quantitatively using Pikovskaya medium. In this study, the phosphate solubilizing bacteria, *Pseudomonas aeruginosa* (strain PS1), *Enterobacter asburiae* (strain PS2), *Pseudomonas putida* (strain PS9) and *Klebsiella* sp. (strain PS19) were used due to their inherent ability to tolerate the highest concentration of pesticides and production of PGP substances in maximum amounts. All these strains produced a largest zone of P solubilization around their growth on solid Pikovskaya medium devoid of pesticides whose solubilization index (SI) ranged between 2 (*Pseudomonas aeruginosa* PS1) and 2.5 (*Klebsiella* sp. PS19). In contrast, the zone of solubilization and *in vitro* solubilization of tri-calcium phosphate (TCP) decreased substantially when PGPR strains were grown with 3X concentrations each of herbicides, insecticides and fungicides. For example, quizalafop-p-ethyl decreased the solubilization zone by 25, 73, 58 and 45%, pyriproxyfen by 38, 50, 43 and 40% and tebuconazole by 25, 63, 58 and 60% at 3X for *Pseudomonas aeruginosa* PS1, *Enterobacter asburiae* PS2, *Pseudomonas putida* PS9 and *Klebsiella* sp. PS19 respectively, over their control. Similar to the effects of pesticides on solubilizing zones, a maximum reduction in P solubilization in broth was found as 96, 82 and 96% by *Pseudomonas aeruginosa* PS1, 95, 87 and 94% by *Enterobacter asburiae* PS2, 95, 93 and 95% by *Pseudomonas putida*

PS9 and 97, 96 and 95% by *Klebsiella* sp. PS19 at 3X of quizalafop-p-ethyl, pyriproxyfen and tebuconazole respectively, over their respective control.

Soils contaminated with pesticides present a major concern for sustainable agriculture. In addition, legumes are used as a rich source of protein in Indian dietary systems, and hence, understanding the effects of pesticides on the legume productivity will be useful. Therefore, the phytotoxic effects of the recommended (X), two (2X) and three (3X) times more of recommended rates of technical grade herbicides (quizalafop-p-ethyl and clodinafop), insecticides (fipronil and pyriproxyfen) and fungicide (tebuconazole) on the biological and chemical characteristics of chickpea, pea, lentil and greengram in pot trials was studied. The rhizobial strains *Mesorhizobium* MRC4, *Rhizobium* MRP1, *Bradyrhizobium* MRM6, *Rhizobium* MRL3 and phosphate solubilizing bacterium *Pseudomonas aeruginosa* PS1 resistant to herbicides (quizalafop-p-ethyl, clodinafop, metribuzin, and glyphosate), insecticides (fipronil, pyriproxyfen, imidacloprid, and thiamethoxam) and fungicides (tebuconazole, hexaconazole, metalaxyl and kitazin) and producing the plant growth promoting substances substantially even in pesticide stress, were used to determine their bioremediation potential using chickpea, pea, lentil and greengram as test crop, when grown in the soil treated with or without herbicides, insecticides and fungicides.

The length of plant organs (roots and shoots) of chickpea grown in sandy clay loam soil treated with the recommended, two and three times more of recommended rates of technical grade herbicides (quizalafop-p-ethyl and clodinafop), insecticides (fipronil and pyriproxyfen) and fungicide (tebuconazole) was measured at 90 and 135 days after sowing (DAS). Generally, a progressive decline with variable magnitude was observed for both roots and shoots length as the concentration of all pesticides was increased from X to 3X in soil. For example, quizalafop-p-ethyl at 3X (120 µg/ kg soil) displayed the most toxic effect and decreased roots length and shoots length by 72 and 53%, respectively (at 90 DAS) and by 73 and 55%, respectively (at 135 DAS) over control. A considerable enhancement was observed in roots and shoots length of inoculated chickpea plants when compared with the uninoculated plants grown in soils treated with the similar concentration of pesticides. For example, when strain MRC4 of *Mesorhizobium* was used with 3X of quizalafop-p-ethyl, it increased the root and shoot length by 42 and 12%, respectively (at 90 DAS) and 22 and 17%, respectively (at 135 DAS) compared with the uninoculated plants grown in soil treated with the same dose of quizalafop-p-ethyl. The

phytotoxicity of pesticides to dry biomass production by plant organs (roots and shoots) and total dry matter accumulation in chickpea plants consistently decreased with increasing concentrations of herbicides, insecticides and fungicides when applied separately. In general, three concentrations each of X, 2X and 3X of all pesticides significantly ($P \leq .05$) decreased the dry matter accumulation both at 90 DAS and 135 DAS, relative to the control. For example, at recommended dose, tebuconazole (100 µg/ kg soil) reduced the root and shoot dry biomass by 48 and 63%, respectively (at 90 DAS) and by 29 and 53%, respectively (at 135 DAS) over control. However, *Mesorhizobium* strain MRC4 increased the shoot dry matter by 30, 28 and 15% at 90 DAS and 82, 16, 14% at 135 DAS at three times of the recommended rates of quizalafop-p-ethyl, fipronil and tebuconazole respectively, compared to uninoculated plants grown in soil treated with the same dose of quizalafop-p-ethyl, fipronil and tebuconazole.

Nodulation response to the three concentrations of quizalafop-p-ethyl, clodinafop, fipronil, pyriproxyfen and tebuconazole at 90 and 135 DAS varied considerably. Generally, each pesticide decreased the nodule numbers and its biomass when concentration of pesticide was increased from normal to three times more of recommended rates. Like plant dry matter accumulation, quizalafop-p-ethyl among herbicides, showed highest toxicity at three times of recommended dose (120 µg/ kg soil) and decreased nodule numbers by 67 and 65% and nodule biomass by 87 and 79% at 90 and 135 DAS, respectively, compared to control. Similarly, pyriproxyfen (insecticide) at 1300 µg/ kg soil (X), 2600 µg/ kg soil (2X) and 3900 µg/ kg soil (3X) adversely affected the chickpea-*Mesorhizobium* symbiosis and decreased nodule numbers by 5, 10 and 14% and nodule mass by 24, 34 and 42% respectively, above the control at 90 DAS. Tebuconazole, at X (100 µg/ kg soil), 2X (200 µg/ kg soil) and 3X (300 µg/ kg soil, also severely affected nodulation and reduced nodule numbers by 24, 34 and 48% and nodule mass by 36, 47 and 56% respectively, at 90 DAS while 3X of the same fungicide declined nodule numbers and nodule dry biomass by 36% and 73% respectively, at 135 DAS compared to control. It was interesting to observe that bioinoculant, in general, significantly ($P \leq 0.05$) improved the nodulation on chickpea plants when grown even in the presence of each class of pesticides. As an example, when strain MRC4 was used with pyriproxyfen at 1300 µg/ kg soil (X), 2600 µg/ kg soil (2X) and 3900 µg/ kg soil (3X), it increased the nodule numbers by 14, 31 and 16% and nodule dry mass by 25, 34 and 42% at 90 DAS while at 135 DAS, it enhanced nodule numbers by 162, 233 and 183% and nodule biomass by 52, 64 and 75%, respectively. The

leghaemoglobin and total chlorophyll content consistently declined with increasing rates of pesticides either in the presence or absence of inoculant and was significant for all pesticides. At highest concentration (3X) of herbicides added to soil, 120 µg/ kg soil of quizalafop-p-ethyl and 1200 µg/ kg soil of clodinafop decreased leghaemoglobin equally by 93% and chlorophyll by 34% and 13% respectively, above control. In addition, pyriproxyfen at 3X (3900 µg/ kg soil) decreased leghaemoglobin and chlorophyll content most severely by 77 and 16%, respectively, which was followed by fipronil that reduced leghaemoglobin and chlorophyll content by 85 and 21%, respectively, over control. In contrast, tebuconazole decreased leghaemoglobin and chlorophyll content by 93 and 28%, respectively, over control. Interestingly, strain MRC4 with 3X of quizalafop-p-ethyl (120 µg/ kg soil), clodinafop (1200 µg/ kg soil), fipronil (600 µg/ kg soil), pyriproxyfen (3900 µg/ kg soil) and tebuconazole (300 µg/ kg soil) increased leghaemoglobin and chlorophyll content by 0% and 45%, 130% and 26%, 133% and 32%, 150% and 32% and 100% and 37%, respectively, compared to uninoculated plants treated with the same dose of quizalafop-p-ethyl, clodinafop, fipronil, pyriproxyfen and tebuconazole, respectively.

Nitrogen (N) and phosphorus (P) content in roots and shoots, seed yield (SY) and grain protein (GP) of chickpea plants was measured at harvest (135 DAS). The measured parameters decreased progressively with increase in the concentration of each pesticide. For instance, at three times of recommended dose, the percent decrease in root N, shoot N, root P, shoot P, SY and GP in presence of clodinafop (at 1200 µg/ kg soil) was 23, 15, 36, 29, 38 and 7, respectively, compared to the control. In contrast, the inoculated strain (MRC4) significantly increased the root N, shoot N, root P, shoot P, SY and GP at all concentration of pesticides relative to uninoculated plants treated with the same dose of pesticides.

Pea plants grown in sandy clay loam soil treated with three concentrations each of quizalafop-p-ethyl, clodinafop, fipronil, pyriproxyfen and tebuconazole 90 and 120 DAS showed a variable plant growth. A pattern of progressive decline with variable degree was recorded for both roots and shoots length as the concentrations of three classes of pesticides were increased in soils. The decreasing order of phytotoxicity of pesticides on the length of plant organs was: quizalafop-p-ethyl > tebuconazole > pyriproxyfen > fipronil > clodinafop. A substantial improvement was observed in roots and shoots length of pea plants inoculated with strain MRP1 when compared with the uninoculated treatments having the same concentration of pesticides. The dry biomass

of roots and shoots continuously decreased as the concentration of each pesticide was increased. Among herbicides, quizalafop-p-ethyl at recommended dose decreased roots and shoots dry mass by 49 and 52%, respectively. Among insecticides, pyriproxyfen decreased roots and shoots dry mass by 29 and 37%, respectively, while fipronil mediated decline in roots and shoots dry biomass was 18 and 34% respectively, at normal rate at 90 DAS over control. Moreover, reduction in roots and shoots dry biomass by fungicide tebuconazole at X was 38 and 54%, respectively at 90 DAS, relative to control. However, in the presence of bioinoculant, the severity of pesticide generated toxicity on biomass accumulation was substantially decreased. For instance, strain MRP1 when used with 3X of tebuconazole, increased the roots dry matter by 81% and shoots dry mass by 60% at 90 DAS. Substantial variation was observed in nodule numbers and nodule dry mass of pea plants grown in soils amended with three concentrations each of quizalafop-p-ethyl, clodinafop, fipronil, pyriproxyfen and tebuconazole at both 90 and 120 DAS. With increase in the concentration of each pesticide, there was a substantial decrease in nodulation. In case of some pesticides, nodule formation was completely diminished when pea plants were grown in soils treated with higher concentration of pesticides. For example, quizalafop-p-ethyl at recommended rate decreased nodule numbers and nodule dry weight by 75 and 48%, respectively, over the control at 90 DAS while at 120 DAS it completely abolished nodulation. Moreover, bioinoculant MRP1 significantly improved the nodulation on pea plants when grown even in the presence of pesticides. For instance, strain MRP1 with tebuconazole at 2X increased nodule numbers by 11% and nodule dry mass by 47% at 90 DAS compared to uninoculated plants grown in soils treated with same dose of tebuconazole. Leghaemoglobin content in fresh nodules and total chlorophyll content in foliage measured at 90 DAS, progressively decreased with increasing concentration of each pesticide both in the presence or the absence of bioinoculant. Quizalafop-p-ethyl, clodinafop, fipronil, pyriproxyfen and tebuconazole at 3X decreased leghaemoglobin and chlorophyll content by 100 and 16%, 24 and 8%, 36 and 10%, 36 and 15% and 100 and 14% respectively, over control. However, a substantial increase in leghaemoglobin and chlorophyll content was observed when inoculated plants were compared with the uninoculated ones treated with the same concentration of pesticides. For illustration, *Rhizobium* strain MRP1, when used with fipronil (at three times more of recommended dose), increased leghaemoglobin and chlorophyll content by 36 and 19% respectively, compared to uninoculated plants treated with the same dose of fipronil. Nitrogen

and phosphorus content, seed yield and grain protein of pea plants measured at harvest, decreased gradually with increasing concentrations of each pesticide. At three times of recommended dose, the percent decrease in root N, shoot N, root P, shoot P, SY and GP was 24, 36, 39, 36, 50 and 4 for quizalafop-p-ethyl; 15, 29, 24, 18, 14 and 2 for clodinafop; 27, 27, 29, 18, 15 and 2 for fipronil; 27, 20, 29, 25, 15 and 2 for pyriproxyfen and 26, 32, 34, 33, 23 and 3 for tebuconazole, respectively, compared to the control. In contrast, when strain MRP1 was used with pesticides, severity of toxicity of all pesticides on these parameters was less pronounced.

Uninoculated and *Bradyrhizobium* inoculated greengram plants grown in soil treated separately with three concentrations each of quizalafop-p-ethyl, clodinafop, fipronil, pyriproxyfen and tebuconazole showed considerable variation in pot house experiments. The length of roots and shoots of greengram plants declined consistently following increase in concentration of pesticides. However, no significant differences were observed for the measured parameters while comparing the effect of pesticides at 50 or 80 days old greengram plants. For instance, clodinafop at 3X, decreased the root length by 65% at 50 DAS while at 80 DAS, it decreased root length by 64%. Similarly, 3X of clodinafop decreased shoots length by 50% at 50 DAS and 63% at 80 DAS relative to control. For plant growth promoting and pesticide tolerant *Bradyrhizobium* (strain MRM6) and *Pseudomonas aeruginosa* (strain PS1) inoculated plants, the roots and shoots length also decreased continuously with increasing concentration of pesticides but significant enhancement was found in the measured parameters of greengram plants when compared with the uninoculated plants grown in soils treated with the same concentration of pesticides. The dry matter accumulation in roots, shoots and the whole greengram plants were adversely affected in response to pesticide application. Generally, all concentrations of pesticides significantly ($P \leq 0.05$) decreased the dry matter accumulation of whole greengram plants both at 50 and 80 DAS, relative to control. For example, tebuconazole at recommended rate decreased roots and shoots dry mass by 16% and 26% respectively, at 50 DAS. The pesticidal toxicity onto greengram plants increased in the order: quizalafop-p-ethyl > tebuconazole > pyriproxyfen > fipronil > clodinafop. Moreover, roots and shoots dry mass at each dose rate of all pesticides increased appreciably when inoculated plants were compared to the uninoculated ones. For example, pesticide tolerant *Bradyrhizobium* strain MRM6 when applied with X, 2X and 3X of clodinafop, significantly ($P \leq 0.05$) increased the root dry matters by 47, 60 and 68% respectively, and shoot dry weight by 48, 55 and 61% respectively, at 50 DAS. While at 80 DAS,

it enhanced the dry matter accumulation in roots by 50, 58 and 62% respectively and shoots dry weight by 7, 13 and 24% at X, 2X and 3X of clodinafop, compared to uninoculated greengram plants grown in soils treated with the same concentration of clodinafop. Furthermore, *Pseudomonas aeruginosa* strain PS1 with 3X of pyriproxyfen dramatically increased the roots dry matter by 247% and shoots dry weight by 413% at 50 DAS while at 80 DAS, it increased roots dry biomass by 447% and shoot dry weight by 513% compared to uninoculated greengram plants treated with the same concentration of pyriproxyfen.

With increasing concentrations of each pesticide, nodule numbers and their dry weight decreased progressively in both presence and absence of bioinoculant. For uninoculated plants, quizalafop-p-ethyl, clodinafop, fipronil, pyriproxyfen and tebuconazole at recommended dose decreased nodule numbers by 29, 10, 10, 15 and 17%, while nodule dry weight by 38, 8, 13, 24 and 25% respectively, at 50 DAS relative to control. In general, quizalafop-p-ethyl displayed the most lethal effect on nodulation. Moreover, bioinoculant strain MRM6 increased the nodule numbers and their mass extensively at all concentrations of each pesticide. For instance, pyriproxyfen at 3X increased nodule numbers and nodule dry mass by 33 and 172% respectively, at 50 DAS while at 80 DAS by 62 and 153% respectively, when compared to the uninoculated plants grown in soils treated with 3X of pyriproxyfen. Also, *Pseudomonas aeruginosa* strain PS1 with clodinafop at 3X increased nodule numbers by 156 and nodule dry mass by 178% at 50 DAS while at 80 DAS, nodule numbers by 63 and nodule dry mass by 293% compared to the uninoculated greengram plants treated with the same dose of clodinafop. Leghaemoglobin and chlorophyll content measured at 50 DAS consistently declined with increasing concentration of each pesticide both in the presence or the absence of the inoculant. For instance, quizalafop-p-ethyl, clodinafop, fipronil, pyriproxyfen and tebuconazole at 3X decreased leghaemoglobin and chlorophyll content by 63 and 25%, 38 and 9%, 38 and 13%, 50 and 14% and 50 and 15% respectively, relative to control. Nevertheless, when *Bradyrhizobium* strain MRM6 when applied with clodinafop at two times more of recommended dose, increased leghaemoglobin and chlorophyll content by 33 and 14% respectively, compared to uninoculated plants at the same dose of clodinafop. Similarly, *P. aeruginosa* strain PS1 with recommended dose of tebuconazole increased leghaemoglobin and chlorophyll content by 14 and 12% respectively, compared to the uninoculated plants raised in soils treated with same dose of tebuconazole. Nitrogen and phosphorus content, seed yield and grain protein measured at 80 DAS decreased regularly with

increasing dose rate of each pesticide both in the presence and the absence of inoculant. For example, at three times of recommended rate, the percent decrease in root N, shoot N, root P, shoot P, SY and GP in the presence of quizalafop-p-ethyl was 45, 44, 52, 37, 63 and 12; 17, 16, 15, 20, 29 and 4 for clodinafop; 34, 22, 23, 14, 38 and 5 for fipronil; 37, 32, 38, 25, 40 and 7 for pyriproxyfen and 25, 30, 38, 34, 49 and 8 for tebuconazole, respectively, compared to control. Interestingly, the inoculant strains (*Bradyrhizobium* strain MRM6 and *P. aeruginosa* strain PS1) significantly ($P \leq 0.05$) increased the root N, shoot N, root P, shoot P, SY and GP at all concentration of pesticides. For instance, *Bradyrhizobium* strain MRM6 when used with 3X of fipronil, increased the root N, shoot N, root P, shoot P, SY and GP by 29, 31, 10, 0, 78 and 5% respectively, compared to the treatment with 3X of fipronil but lacking inoculant. Similarly, *P. aeruginosa* strain PS1 when used with 3X of clodinafop, increased the root N, shoot N, root P, shoot P, SY and SP by 27, 38, 13, 34, 83 and 3%, respectively, when compared to the treatment having the same dose of clodinafop but without inoculant.

All pesticides showed the phytotoxicity and reduced of the length of both roots and shoots of lentil plants progressively, as the concentration of pesticides was increased in soils from recommended to three times more of recommended rate. In general, quizalafop-p-ethyl affected adversely the growth of roots and shoots of lentil plants. Moreover, the effects of other pesticides like fipronil, pyriproxyfen and tebuconazole on root and shoot length was comparable. Substantial increase in root and shoot length of *Rhizobium* strain MRL3 inoculated lentil plants occurred compared to the uninoculated plants treated with the same concentration of pesticides. For example, strain MRL3 in the presence of tebuconazole (at three times of recommended dose), increased the root and shoot length by 43 and 27% respectively, at 90 DAS while at 120 DAS by 8% and 17% respectively, compared to the uninoculated plants treated with the same dose of tebuconazole. At recommended dose, quizalafop-p-ethyl, clodinafop, fipronil, pyriproxyfen and tebuconazole decreased roots and shoots dry biomass by 40 and 45, 10 and 8, 19 and 19, 32 and 24 and 28 and 36% respectively, at 90 DAS while at 120 DAS by 52 and 41, 7 and 6, 22 and 11, 26 and 16 and 32 and 24% respectively, over control. Maximum decline in plant root (68 and 71%) and shoot (65 and 63%) dry matters was shown by quizalafop-p-ethyl (3X) at 90 and 120 DAS, respectively. Effect of other pesticides was however, comparatively less inhibitory. Furthermore, *Rhizobium* strain MRL3 significantly increased the dry biomass at all dose rates of pesticides when inoculated lentil plants were compared to the uninoculated

plants. For example, strain MRL3 with X, 2X and 3X of clodinafop increased the shoots dry matter by 67, 80 and 60% respectively, at 90 DAS while at 120 DAS by 76, 75 and 80%, compared to uninoculated treatments treated with the same dose rates of clodinafop.

Nodulation in lentil plants grown in soils treated with the three concentrations each of quizalafop-p-ethyl, clodinafop, fipronil, pyriproxyfen and tebuconazole at 90 and 120 DAS varied considerably. Like other legume crops tested in this study, the nodule numbers and the dry weight of lentil nodules also declined when concentration of pesticide was increased from X to 3X. A least toxic effect on nodulation was however, shown by clodinafop which at X, 2X and 3X, decreased the nodule numbers by 6, 11 and 37% (at 90 DAS) and by 3, 19 and 27% (at 120 DAS) and nodule dry mass by 7, 20 and 30% (at 90 DAS) and 7, 17 and 32% (at 120 DAS), respectively. Quizalafop-p-ethyl at 3X showed maximum toxicity among pesticides and decreased both nodule numbers and nodule biomass equally by 100 and 64% at both 90 and 120 DAS, respectively. However, the bioinoculant significantly increased the nodule numbers and nodule biomass when compared to uninoculated treatments of the same concentration. For example, *Rhizobium* strain MRL3 with fipronil at X, 2X and 3X, increased nodule numbers by 50, 38 and 50% respectively, and nodule dry mass by 108, 86 and 84% respectively, at 90 DAS while at 120 DAS, nodule numbers by 3, 7 and 11% respectively, and nodule biomass by 23, 36 and 60%, respectively, compared to the uninoculated plants treated with the same dose of fipronil. The leghaemoglobin and total chlorophyll content also consistently decreased with increasing concentrations of pesticides. At recommended dose, quizalafop-p-ethyl, clodinafop, fipronil, pyriproxyfen and tebuconazole decreased leghaemoglobin and chlorophyll content by 100 and 22%, 9 and 4%, 17 and 7%, 25 and 4% and 34 and 13% respectively, relative to control. However, substantial increase in leghaemoglobin and chlorophyll content was observed when inoculated plants were compared with the uninoculated ones grown in soils amended with same concentration of pesticides. Nitrogen and phosphorus content, seed yield and grain protein (GP) of lentil plants measured at harvest (120 DAS) decreased progressively with increasing concentration of each pesticide from X to 3X. However, the inoculated strain significantly increased the root N, shoot N, root P, shoot P, SY and SP at all concentration of pesticides compared to uninoculated plants. For example, *Rhizobium* strain MRL3 when used with pesticides, increased the root N, shoot N, root P, shoot P, SY and SP by 30, 7, 41, 21, 55 and 6%, respectively, at 3X of clodinafop and 33, 8, 61, 26 and 111 and 5%, respectively, at 3X of

tebuconazole when compared to the treatments with the same dose of pesticides but devoid of inoculant. The study thus suggested that the pesticide tolerant rhizobial strains (*Mesorhizobium* strain MRC4, *Rhizobium* strain MRP1, *Bradyrhizobium* strain MRM6 and *Rhizobium* strain MRL3) or phosphate solubilizing strain (*Pseudomonas aeruginosa* strain PS1) due to their intrinsic abilities of growth promotion and attenuation of the toxic effects of pesticides could be developed as inoculant and be exploited for remediation or restoration of pesticide polluted soils.

There has been a tremendous research on enhancing crop productivity through the introduction of PGPR in conventional soils applying different methods to find out both the super-inoculant and the strategies as to how the productivity could be improved. The challenge now is however to develop different strategies to identify pesticide resistant PGPR that may work competently and simultaneously in geographically and agronomically different soils. Furthermore, exploration of novel genes expressing greater potential of degradation or transforming a wide range of agrochemicals including herbicides/insecticides/fungicides among the heterogeneous bacterial communities inhabiting the pesticide stressed environment and enhancing the plant growth promoting efficiency of the pesticide resistant plant growth promoting rhizobacteria through genetic manipulation may provide new insights to upgrade economically feasible and ecologically sound agriculture systems.

EFFECT OF AGROCHEMICALS ON RHIZOTROPHIC MICROFLORA AND LEGUME-*RHIZOBIUM* SYMBIOSIS

THESIS

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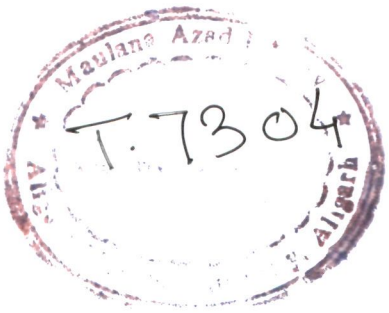

Dedicated to the loving memory of

My Great Father (Late)

Who was not a person but an institution in himself!

and to

My Adoring Mother

Who is self-sacrificing, altruistic, never-tiring and ever-inspiring personality

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CERTIFICATE

This is to certify that the work embodied in this thesis entitled **"Effect of Agrochemicals on Rhizotrophic Microflora and Legume-*Rhizobium* Symbiosis"** has been carried out by **Mr. Munees Ahemad**, under my supervision. The work included in this thesis is original and has not been submitted for any other degree. The work is suitable for the award of Ph. D. degree in Microbiology of Aligarh Muslim University, Aligarh.

Date: 30.10.2009

Dr. Mohd. Saghir Khan
(Supervisor)

October, 19, 2009

DECLARATION

This thesis entitled "**Effect of Agrochemicals on Rhizotrophic Microflora and Legume-*Rhizobium* Symbiosis**" contains no material which has been accepted for the award of any other degree or diploma in any University. To the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference has been made.

Munees Ahemad

(Munees Ahemad)

CONTENTS

List of Abbreviations

PGPR	Plant growth promoting rhizobacteria
iPGPR	Intracellular plant growth promoting rhizobacteria
ePGPR	Extracellular plant growth promoting rhizobacteria
IAA	Indole acetic acid
YEM	Yeast extract mannitol
BNF	Biological nitrogen fixation
ACC	1-aminocyclopropane-1-carboxylate
HCN	Hydrogen cyanide
CAS	Chrome Azurol S
DHBA	2, 3-Dihydroxy benzoic acid
SA	Salicylic acid
PSM	Phosphate solubilizing microorganism
PSB	Phosphate solubilizing bacteria
RP	Rock phosphate
CFU	Colony forming unit
cm	Centimeter
DAS	Days after sowing
rDNA	Ribosomal deoxyribonucleic acid
h	Hour
L	Liter
LSD	Least significant difference
mg	Milligram
ml	Milliliter
M	Molar
mM	Milimolar
N	Nitrogen
nm	Nanometer
s	Second
rpm	Revolutions per minute
ABA	Abscisic acid

P	Phosphate
µM	Micromolar
EPS	Exopolysaccharides
v/v	Volume per volume
kg	Kilogram
g	Gram
X	Recommended dose
MRL	Maximum resistance level
SI	Solubilization index
TCP	Tri-calcium phosphate
±	Standard deviation
Lb	Leghaemoglobin
Chl	Chlorophyll
DAE	Days after emergence
BSA	Bovine serum albumin
ANOVA	Analysis of variance
df	Degree of freedom
-	Negative
%	Percent
°C	Degree centigrade
µg	Microgram
µl	Microlitre
+	Positive

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Munees Ahemad

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Introduction

In high input agricultural practices, the wide spread use of agrochemicals including pesticides has become an important factor for achieving higher crop yields. Many of these applications include a wide array of active ingredients and each of which is, by design, a biocide whose value lies in its ability to kill organisms particularly pests that are injurious and a potential threat for agricultural crop production system. Pests and diseases together cause a 20-40% loss of global crop production (Mazaud, 1997). Moreover, the average world grain loss is estimated to be 20% and these losses may occur at all stages of food production like harvesting, storage, processing and retailing (Hay et al., 1997, Voinova et al., 2009).

To prevent such huge losses, the use of pesticides has emerged as a strong weapon to control and manage plant pests and diseases. Pesticides, toxic by design, are generally, grouped according to their primary target organisms. And hence, several groups of specific, broad spectrum and systemic pesticides have been identified/recognized and developed in recent times. Accordingly, their profound effectiveness has led to an exponential increase in the marketability of pesticides and their application under field environment has increased several folds (Manjunath, 2000). The use of pesticides in agricultural systems has expanded primarily due to: (i) relatively low cost and high returns (ii) the intensification of agriculture, including the introduction of high yielding varieties requiring greater pesticide applications (iii) lack of understanding among users regarding the consequences of pesticide use and (iv) a tendency to try and achieve total protection from pests in high yielding crops across a wide range of agricultural systems. However, the molecular complexity of the pesticides varies greatly which behave very differently in soils.

Among the various agrochemicals, herbicides, insecticides and fungicides are frequently used in agronomic practices to combat the organisms detrimental to crop plants and consequently to increase the crop productivity in different agro-ecosystems. Pulses like other crops are also infested by a large number of pests, such as pod borers, aphids, jassids, and pod flies and plant pathogenic fungi which cause a substantial reduction in crop yields (Mukherjee et al., 2007). Pesticides are, therefore, commonly applied to crop fields in order to circumvent common crop damaging pests and hence, facilitate crop productivity. However, after application, a large portion of pesticides accumulate in the top soil layer (0-10 cm) and exert deleterious effects on structures and functions of microbial communities (Fabra et al., 1998, Das et al., 2005, Ahmed and Ahmad, 2006, Pampulha and Oliveira, 2006, Pampulha et al., 2007, Virág et al., 2007)

thereby leading to a loss in soil fertility (Pal et al., 2006). In real soil systems, microbial communities are heterogeneously distributed and play an active role in cycling of elements in ecological systems and determine the nutrient pool of soils which in turn, impact the growth and development of plants (Khan et al., 2009b, Qi et al., 2009).

A well established practice for maintaining soil fertility has been the cultivation of legume crops, which replenish nitrogen in the soil by forming symbiosis with N_2 fixing bacteria, like *Rhizobium*, *Mesorhizobium* and *Bradyrhizobium* etc. that convert atmospheric N to ammonia and other compounds and transport it to growing plants. The effectiveness of this strategy however, relies largely on maximizing symbiotic N_2 fixation (SNF) and plant yield to resupply organic and inorganic N and nutrients to the soil (Fox et al., 2007). And hence, in order to increase the N pool of soils, rhizobial inoculants are commonly applied to soils/seeds of legumes to ensure effective nodulation and subsequent N_2 fixation (Dudeja and Singh, 2008). The inoculant is, however, often used in conjunction with agrochemicals, which besides containing essential nutrients also contain contaminants and toxic elements. The exposure of these chemicals to field-grown plants could be either intentional (e.g. by spraying the legumes with pesticides) or through residues remaining from previous applications (Khan et al., 2004). Of these chemicals, the persistence of pesticides into soils may be potentially hazardous and cause a threat to both legumes and associated symbiotic nitrogen-fixing organisms. The common use of pesticides in agricultural practices has been shown to adversely affect N_2 fixation, either directly by affecting the rhizobia (Mallik and Tesfai, 1985, Anderson et al., 2004) and disrupting the signaling between legume-derived phytochemicals (luteolin, apigenin) and *Rhizobium* Nod D receptors (Fox et al., 2007), or indirectly by reducing photosynthate allocation to the nodules for N_2 fixation (Sprout et al., 1992, Koopman et al., 1995, Datta et al., 2009) or by restricting root growth and hence reduce the number of sites available for infection (Eberbach and Douglas, 1991). Additionally, pesticides that persist in soils may have a long-lasting impact on rhizobial survival and function (Eberbach and Douglas, 1989, Mårtensson and Nilsson, 1989, Eliason et al., 2004). These observations thus warrants a rigorous screening of all the pesticides used either at recommended rates or rates higher than the recommended one in order to get the full benefit of pesticide applications under field conditions.

To overcome the pesticide stress, microorganisms of agricultural importance including plant growth promoting rhizobacteria (PGPR) and N_2 fixers have evolved a number of

mechanisms which they use to tolerate the high concentration of pesticides (Singh et al., 2006). Such reduction in pesticidal toxicity by microbes may be through biodegradation (Wang et al., 2008, Hsiao et al., 2007) or biotransformation i.e. transformation of toxic chemicals to less toxic forms (Parales et al., 2002). Due to these traits, when plant growth promoting rhizobacteria as seed bioinoculant are applied to soil treated with pesticides either intentionally or previously contaminated, have shown a substantial reduction in the toxicity of pesticides to plants and concurrently resulting in improved growth and yield of plants (Upadhyay and Sharma, 2003, Radha et al., 2009, Rinu and Pandey, 2009). As an example, three bacterial strains tolerant to glufosinate (herbicide), identified by 16s rDNA analysis as *Burkholderia sacchari*, *Serratia mercescens* and *Pseudomonas psychrotolerans* when grown in culture medium and soil containing high concentration of glufosinate have shown a significant ability to degrade this glutamine synthetase inhibitor suggesting that glufosinate-degrading bacteria could be isolated from soils, after a long term induction or selection. Furthermore, this study suggests that a long term exposure of herbicides is a promotive factor in generating bacterial strains having high degradation efficiency and showing vigorous propagation under the competitive pressure arising from indigenous microbes (Hsiao et al., 2007).

Rhizospheric plant growth promoting bacteria besides reducing pesticidal toxicity are also known to play a pivotal role in augmenting the crop yields employing direct or indirect mechanisms in both conventional and derelict soils (Khan et al., 2009a). The direct mechanisms of plant growth promotion involve the production of substances by rhizobacteria or facilitation of the uptake of nutrients from the recipient environment (Glick et al. 1999, Wani et al., 2007a, Wani et al., 2008). The plant growth promoting bacteria in general, enhance the growth of plants directly by i) increasing N uptake by legume plants through biological N_2 fixation (Hara et al., 2009, Radha et al., 2009) ii) increasing the availability of solubilized forms of P to plants through solubilization of insoluble phosphate compounds (Khan et al., 2009a, Wani et al., 2007b) iii) sequestering iron by production of different types of siderophores (Wani et al., 2007a, Wani et al., 2008) iv) production of phytohormones such as auxins, cytokinins, gibberellins (Singh et al., 2008, Selvakumar et al., 2008) v) alleviating the stress induced by ethylene on plants by synthesizing 1-aminocyclopropane-1-carboxylate (ACC) deaminase (Shaharoona et al., 2008, Indiragandhi et al., 2008) and vi) reducing the toxicity of pesticides through degradation to non toxic compounds (Bano and Musarrat, 2003, Shivaramaiah and Kennedy,

2006). The indirect promotion of plant growth on the other hand, occurs when these rhizobacteria lessen or prevent the injurious effects of plant pathogens by production of inhibitory substances or by increasing the natural resistance of the host plants (Saravanakumara et al., 2007, Cazorla et al., 2007, Vasileva and Ilieva, 2007).

Pulses are important source of dietary proteins, and have unique property of maintaining and restoring soil fertility through biological N_2 fixation as well as conserving and improving physical properties of soil by virtue of their deep root system and leaf fall. Pulse crops add a reasonable quantity of nitrogen (upto 30 Kg N/ha) to soils. Of the different legumes grown around the world, chickpea (*Cicer arietinum*), pea (*Pisum sativum*), greengram (*Vigna radiata* L. wilczek) and lentil (*Lens esculentus*) are the most widely grown legumes. In India, chickpea occupies 7.1 million ha with a production of 5.75 million tones, accounting for 31% and 31% of total pulse area and production, respectively; greengram occupies 3 million ha and contributing to 1.3 million tones pulse production; lentil occupies 1.34 million ha and contributes 0.88 million tones to pulse production and pea is cultivated on 0.73 million ha with annual production of 0.72 million tones (ICAR, 2006). Due to inadequate and conflicting reports on the toxicity of pesticides on plant growth promoting rhizobacteria and legume-*Rhizobium* symbiosis and the possibility of damage to the legumes due to the application of pesticides into the soils, it was desirable to explore the diversity of plant growth promoting rhizobacteria in terms of their functional variation in Aligarh district of Western Uttar Pradesh, India. Subsequently, the toxicity of certain pesticides to the functional properties of selected PGPR and the effect of pesticides and pesticide tolerant PGPR strains on popularly grown legumes in the region was also investigated. The present investigation was therefore, designed with specific objectives:-

1. To assess soil microbial diversity in different rhizospheres of popularly grown crops grown in this area.
2. To isolate nitrogen fixing bacteria from the nodules of legumes, chickpea, pea, greengram and lentil and phosphate solubilizing bacteria from mustard rhizosphere.
3. To evaluate the tolerance of rhizobacteria (nitrogen fixers and phosphate solubilizing bacteria) and growth pattern against selective herbicides (quizalafop-p-ethyl, clodinafop, metribuzin and glyphosate), insecticides (fipronil, pyriproxyfen, imidacloprid and thiamethoxam) and fungicides (tebuconazole, hexaconazole, metalaxyl and kitazin).

4. To assay both qualitatively and quantitatively the production of plant growth promoting substances by PGPR strains.
5. To assess the *in vitro* biotoxicity of pesticides to plant growth promoting traits of both nitrogen fixers and phosphate solubilizers and to identify pesticide tolerant strains for their PGP activities under pesticide stressed condition.
6. To evaluate the phytotoxic effects of recommended and higher doses of selective pesticides including herbicides (quizalafop-p-ethyl and clodinafop), insecticides (fipronil and pyriproxyfen) and a fungicide (tebuconazole) on the biological and chemical properties of chickpea, greengram, lentil and pea preferably grown in the vicinity of Aligarh.
7. To evaluate the effect of the pesticide tolerant plant growth promoting rhizobacteria on the performance of chickpea, greengram, lentil and pea plants grown in pesticide stressed soils.

Literature Review

2.1. Rhizosphere

The complexity of the soil ecosystem is established by the numerous and diverse interactions among its physical, chemical, and biological components (Buscot, 2005). Especially, the variable genetic and functional activities of the heterogeneous microbial populations have a vital effect on soil functions, as such microbes are considered powerful forces for specific enzyme mediated fundamental metabolic processes (Nannipieri et al., 2003). Moreover, these microorganisms affect the biogeochemical cycling of nutrients and consequently help plants to grow better both under conventional and stressed soil environment (Barea et al., 2004, Wani et al., 2007a, Wani et al., 2008, Khan et al., 2009b). Microbial communities inhabiting soils interact with plant roots and soil constituents at the root–soil interface (Glick, 1995; Barea et al., 2002a, b). And hence, the rhizosphere can be defined as any volume of soil specifically influenced by plant roots and/or in association with roots hairs, and plant-produced materials (Dessaux et al., 2009). Largely, three separate but interacting components are recognized in the rhizosphere: the rhizosphere (soil), the rhizoplane, and the root itself. Of these, the rhizosphere is the zone of soil influenced by roots through the release of substrates that affect microbial activity. The rhizoplane, on the other hand, is the root surface, including the strongly adhering soil particles (Fig. 1) while the root itself is a part of the system, because certain micro-organisms (like endophytes) colonize root tissues (Bowen and Rovira, 1999). The unique physico-chemical and biological characteristics of the soils that are associated with roots, compared to the soils away from the root and root surface are responsible for the enhanced microbial diversity together with increased numbers and activity of microorganisms in the rhizosphere (Kennedy, 1998). During microbes-plant interaction, plant roots exude the organic materials which are used up by root associated microorganisms as nutrients as well as organic material is also supplied to the soil micro biota through the death and decay of plants as either growth substrates, structural components or signal molecules. Microbial activity in the rhizosphere affects the rooting patterns and the supply of available nutrients to plants, in a manner that modify the quality and quantity of root exudates (Azcon, 1987, Barea, 2000, Gryndler, 2000).

2.2. Plant growth promoting rhizobacteria

Microorganisms are a fundamentally important component of the soil habitat where they play key roles in ecosystem functioning through controlling nutrient cycling reactions essential

for maintaining soil fertility and also contributing to the genesis and maintenance of soil structure (Kirk et al. 2004). The term rhizobacteria is used to describe a subset of rhizosphere bacteria capable of colonizing the root environment (Kloepper et al., 1991, Kloepper, 1994). Beneficial, root colonizing, rhizosphere bacteria, the PGPR (plant growth promoting rhizobacteria), are defined by three intrinsic characteristics: (i) they must be able to colonize the root (ii) they must survive and multiply in microhabitats associated with the root surface, in competition with other microbiota, at least for the time needed to express their plant growth promotion/protection activities, and (iii) they must promote plant growth. Plant growth-promoting rhizobacteria are thus free-living, soil-borne bacteria which, when applied to seeds/soils or crops, enhance the growth of the plant directly by providing nutrients to plants or indirectly by reducing the damage from soil-borne plant pathogens (Kloepper et al., 1980).

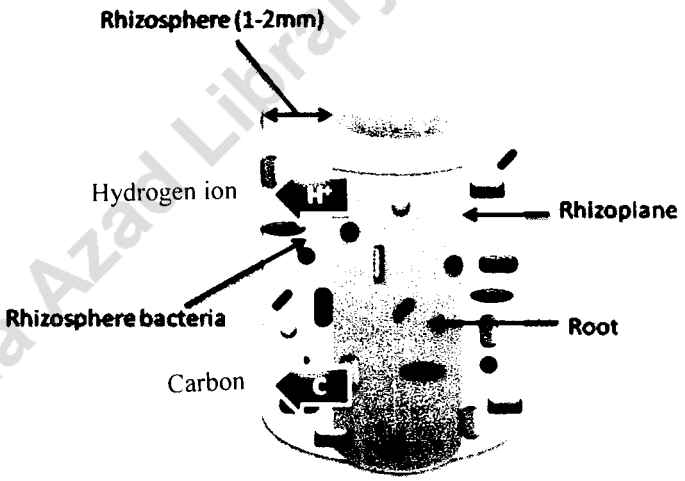


Fig. 1: Schematic representation of rhizosphere (modified from Vega, 2007)

The PGPR belonging to various bacterial genera are known to participate in many important biological activities, such as the biological control of plant pathogens, nutrient cycling, and/or seedling growth (Persello-Cartieaux et al., 2003; Zahir et al., 2004) through the production of various substances (Table 1). Among PGPR, *Pseudomonas* and *Bacillus* are the most commonly described genera possessing plant growth promoting activities, but many other taxa are also included in PGPR group. Selected strains of PGPR are being used as seed inoculant (Zahir et al., 2004, Sahin et al., 2004, Zaidi and Khan, 2006, Wani et al., 2007b, Rani et al., 2009)

In general, PGPR can be divided into two categories (i) extracellular PGPR (ePGPR), existing in the rhizosphere, on the rhizoplane or in the spaces between cells of the root cortex, and (ii) intracellular PGPR (iPGPR), which exist inside root cells, generally in specialized nodular structures. The latter includes rhizobia and *Frankia* species, both of which fix N in symbiosis with higher plants (Gray and Smith, 2005). Functionally, the PGPR have been separated into two groups: (i) those involved in nutrient cycling and phytostimulation, (direct activity) and (ii) those involved in the biocontrol of plant pathogens (indirect activity) (Bashan and Holguin, 1998).

Table 1 Growth promoting substances released by selected PGPR

PGPR	Plant growth promoting traits	References
Mesorhizobium sp.	IAA, siderophores, HCN, ammonia	Wani et al. (2008)
Azospirillum amazonense	IAA, nitrogenase activity	Rodrigues et al. (2008)
Proteus vulgaris KNP3	Siderophores	Rani et al. (2009)
Mesorhizobium ciceri RC4, Azotobacter chroococcum A10	IAA, siderophores	Wani et al. (2007c)
Pseudomonas PSB5, *Bacillus* PSB9	Phosphate solubilization, IAA and siderophores	Wani et al. (2007c)
Klebsiella oxytoca	IAA, phosphate solubilization, nitrogenase activity	Jha and Kumar (2007)
Bacillus spp., *Pseudomonas* spp., *Azotobacter* spp., *Rhizobium* spp.	IAA, ammonia production	Joseph et al. (2007)
Bradyrhizobium sp.	IAA, siderophores, HCN, ammonia	Wani et al. (2007a)
Rhizobium sp.	IAA, siderophores, HCN, ammonia	Wani et al. (2007b)
Pseudomonas fluorescens	Induced systemic resistance, antifungal activity	Saravanakumara et al. (2007)
Pseudomonas chlororaphis	Antifungal activity	Liu et al. (2007)
Acinetobacters sp., *Pseudomonas aeruginosa*, *Staphylococcus saprophyticus*, *Bacillus cereus*, *Enterobacter hormaechei*, *Pantoae agglomerans*, *Alcaligenes faecalis*	IAA, HCN, lipase and protease production	Egamberdieva et al. (2007)
Bacillus subtilis	Antifungal activity	Cazorla et al. (2007)
Gluconacetobacter diazotrophicus	Zinc solubilization	Saravanan et al. (2007)
Brevibacillus spp.	Zn resistance, IAA	Vivas et al. (2006)
Pseudomonas putida KNP9	Siderophores, Pb and Cd resistence	Tripathi et al. (2005)
Pseudomonas fluorescens	IAA, siderophores, antifungal activity	Dey et al. (2004)
Azospirillum brasilense, *Azospirillum amazonense*	IAA, P solubilization, nitrogenase activity, antibiotic resistance	Thakuria et al. (2004)
Pseudomonas fluorescens	IAA, phosphate solubilization	Jeon et al. (2003)
Pseudomonas sp. NBRI 4014	IAA, siderophores, phosphate solubilization	Gupta et al. (2002)
Pseudomonas fluorescens	Antifungal activity	Botelho et al. (1998)
Kluyvera ascorbata	ACC deaminase, siderophores, metal resistance	Genrich et al. (1998)

2.2.1. Root colonization by PGPR

The distribution, colonization, multiplication and establishment of introduced PGPR are profoundly affected by biotic and abiotic factors. The optimal temperature for growth of many

PGPR *in vitro* is above 25 °C but root colonization is generally greatest below 20 °C (Loper et al., 1985). Better root colonization at lower temperature probably reflects the fact that microbial activity in the soil declines with temperature. Further, slower root growth at lower temperatures may facilitate more effective transport of the bacteria from the inoculum source to the roots. Although PGPR grow best *in vitro* at neutral pH or above, colonization is better at lower pH (Edwards et al., 1998).

The biological composition of the rhizosphere dramatically influences root colonization. Accordingly, the nutrient availability rather than space is the primary determinant of microbial population size in the rhizoplane and rhizosphere (Pal et al., 1998). Thus, inoculation of PGPR does not lead to a change in the total rhizosphere population, but a shift in the composition of the microflora such that introduced bacteria preempts establishment of the normal indigenous strains. The root colonization hence will be greater in sterile or pasteurized soils than in raw soil due to lack of competition, antibiosis and predation from the natural inhabitants of soils. In contrast, as microbial activity increases in unsterilized soils, through inputs of nutrients, the level of colonization by introduced PGPR is reduced (Casida, 1992). Pathogens that are targets of PGPR can influence PGPR populations either positively or negatively (Edwards et al., 1998).

Root colonization is a multistage event involving many bacterial traits and genes. Adhesion of PGPR to roots may be either non-specific resulting from electrostatic forces or involve glycoprotein termed agglutinin (Anderson et al., 1988). For instance, Buell and Anderson (1992) characterized a locus, *agg* A, from *Pseudomonas* that encodes a 50.5 KDa protein required for agglutinability and adherence. Similarly, several exopolysaccharides (EPS) are reported to be involved in the attachment of rhizobacteria to plant cells and in the nodulation of legumes by *Rhizobium* (Cangelosi et al., 1987). However, one approach to increasing root colonization by PGPR is to increase the level of bacterial inocula applied to the seeds (Bull et al., 1991). Enhancement in colonization by increasing the initial dose of bacteria on the seeds has, however, limitations (Osburn et al., 1989). Another strategy to increase the colonization involves the use of multiple bacterial strains; PGPR research has focussed primarily on the use of single strains. However, Weller and Cook (1983) reported that the use of *P. fluorescens* 2-79 in combination with *P. fluorescens* 13-79 was superior to either strain when used alone in about 50% of the trials. Furthermore, recombinant DNA technology has provided the most exciting and potentially

successful means to improve root colonization and biological control by PGPR (Natsch et al., 1997).

2.2.2 Agricultural importance of PGPR

The use of PGPR to augment crop productivity has been limited largely due to the variability and inconsistency of results observed under laboratory, greenhouse and field trials. Soil is an unpredictable environment and an intended result is sometimes difficult to achieve. Climatic variations has also a large impact on the effectiveness of PGPR but sometimes unfavorable growth conditions in the field are to be expected as a normal functioning of agriculture. Despite all these factors, increase in crop yields following PGPR applications in the growth chambers and field trials have also been observed (Mishra and Goel, 1999, Lucy et al., 2004, Zaidi and Khan, 2006, Wani et al., 2007c) as presented in Table 2.

2.2.3. How PGPR facilitate plant growth?

The PGPR strains facilitate growth of plants either directly or indirectly (Fig. 2) (Glick, 1995). The direct mechanism of plant growth promotion involves the production of substances by bacteria and its transport to the developing plants or facilitates the uptake of nutrients from the recipient environment (Azcon, 1989). The direct growth promoting activity of PGPR includes-(i) N_2 fixation (Wani et al., 2007c) (ii) solubilization of insoluble phosphorus (Khan et al., 2006c, Khan et al., 2009a) (iii) sequestering of iron by production of siderophores (Wani et al., 2008, Rajkumar et al., 2006) (iv) production of phytohormones such as, auxins, cytokinins, gibberellins and (v) lowering of ethylene concentration (Liu et al., 2007, Rodrigues et al., 2008; Wani et al., 2008). On the contrary, the indirect mechanism of plant growth promotion by PGPR includes (i) antibiotic production (ii) depletion of iron from the rhizosphere (iii) synthesis of antifungal metabolites (iv) production of fungal cell wall lysing enzymes (v) competition for sites on roots and (vi) induced systemic resistance (Saravanakumara et al., 2007, Cazorla et al., 2007). Briefly, the indirect promotion of plant growth takes place when PGPR lessen or prevent the injurious effects of plant pathogens by synthesizing inhibitory substances or by increasing the natural resistance of the host to the pathogens.

2.2.3.1. Direct mechanisms of action

2.2.3.1.1. Nitrogen fixation

Nitrogen (N) is one of the most common nutrients required for optimal plant growth and productivity. Even though, more than 78% of N is present in the atmosphere, yet it remains unavailable to growing plants. It therefore, needs to be converted into ammonia, an available form to plants and other eukaryotes. Atmospheric N is converted into plant utilizable forms by three different processes: (a) conversion of atmospheric N into oxides of N in the atmosphere (b) industrial N fixation that involves the use of catalysts and high temperature (300-500 °C) to transform N into ammonia and (c) biological N fixation (BNF) which changes the nitrogen to ammonia by microorganisms using a complex enzyme system known as nitrogenase (Kim and Rees, 1994). Of all the processes, BNF fixes about 60% of the earth's available N and represents an economically beneficial and environmentally sound alternative to chemical fertilizers (Ladha et al., 1997).

Nitrogen fixing organisms can broadly be divided as- (a) symbiotic N_2 fixing bacteria, that includes members of family rhizobiaceae and forms symbiosis with leguminous host plants (e.g. rhizobia) (Zahran, 1991, Zahran, 2001) and non-leguminous trees (e.g. *Frankia*). Gram-negative soil bacteria (rhizobia) within the rhizobiaceae phylogenetic family (α-proteobacteria) possess the unique ability to infect and establish a nitrogen-fixing symbiosis with the roots of leguminous plants. The establishment of the symbiosis involves a complex interplay between host and symbiont (Giordano and Hirsch, 2004) resulting in the formation of a novel organ, the nodule, which the bacteria colonize as intracellular symbionts (b) non-symbiotic (free living, associative and endophytes) nitrogen fixing forms such as cyanobacteria (*Anabaena*, *Nostoc*), *Azospirillum*, *Azotobacter*, *Gluconoacetobacter diazotrophicus* and *Azocarus* etc. Plant growth-promoting rhizobacteria that fix N_2 in non-leguminous plants are also called as diazotrophs capable of forming a non-obligate interaction with the host plants (Glick et al., 1999). The process of N_2 fixation is carried out by the nitrogenase enzyme coded by *nif* genes (Kim and Rees, 1994). Nitrogenase was elucidated by Dean and Jacobson (1992) as a two-component metalloenzyme consisting of (i) dinitrogenase reductase which is the iron protein and (ii) dinitrogenase which has a metal cofactor. Based on the metal cofactor three different N fixing systems have been identified (a) Mo-dinitrogenase, (b) V-nitrogenase and (c) Fe-nitrogenase. The existence of the N_2 fixing system differs among bacterial genera (Bishop and Jorerger, 1990).

Table 2 Examples of plant growth promoting rhizobacteria tested for various crop types

PGPR	Plant	Conditions	Results of addition of bacteria to plants	Reference
Pseudomnas putida strain R-168, *Pseudomonas fluorescens* strain R-93, *Pseudomonas fluorescens* DSM 50090, *Pseudomonas putida* DSM291, *Azospirillum lipoferum* DSM 1691, *Azospirillum brasilense* DSM 1690	Maize (*Zea mays* L.)	Fields	Plant height, seed weight, number of seed per ear and leaf area, shoot dry weight significantly increased	Gholami et al. (2009)
Azotobacter chroococcum, Azospirillum lipoferum	Cotton (*Gossypium hirsutum* L.)	Fields	Seed yield (21%), plant height (5%) and microbial population in soil (41 %) increased over their respective controls while boll weight and staple length remained statistically unaffected	Anjum et al. (2007)
Pseudomonas putida CC-R2-4, *Bacillus subtilis* CC-pg104	*Lectuca sativa* L.	Gnotobiotic conditions	Significant increase in shoot length and root length achieved through encapsulated inoculant	Rekha et al. (2007)
Azospirillum spp., *Rhizobium* spp.	Genotypes, BAT477 and DOR364 of common bean (*Phaseolus vulgaris* L.)	Fields	*Azospirillum–Rhizobium* co-inoculation as compared to single *Rhizobium* inoculation increased the fixed N and the yield of DOR364 across all sites, on the contrary, for BAT477, , a negative effect of *Azospirillum–Rhizobium* co-inoculation on the same parameters was observed	Remans et al. (2008a)
Azospirillum amazonense	Rice (*Oryza sativa* L.).	Greenhouse	Grain dry matter accumulation (7 to 11.6%), the number of panicles (3 to 18.6%) and nitrogen accumulation at grain maturation (3.5 to 18.5%) incresed	Rodrigues et al. (2008)
Azospirillum brasilense Sp245	Wheat	Greenhouse	Increased growth considerably	Spaepen et al. (2008)
Pseudomonas strains Pf1, TDK1, PY15	Rice	Fields, greenhouse	disease resistance in rice plants against sheath rot (*Sarocladium oryzae*) disease	Saravanakumar et al. (2008)
Pseudomonas species	Rice (*Oryza sativa*), maize (*Zea mays*)	*In vitro*	that pseudomonad isolated from rice showed a higher ability to control bacterial and fungal root pathogens than that obtained from maize	Lawongsa et al. (2008)
Azospirillum brasilense Sp245	Common bean (*Phaseolus vulgaris* L.)	Greenhouse	Root growth increased	Remans et al. (2008b)
Pseudomonas fluorescens PGPR1, PGPR2, PGPR4	Peanut (*Arachis hypogaea* L.)	Pots, fields	significantly enhanced pod yield, haulm yield and nodule dry weight over the control	Dey et al. (2004)
Rhizobium tropici and *Paenibacillus polymyxa*	Common bean (*Phaseolus vulgaris* L.)	Greenhouse	increased nodulation and nitrogen fixation	Figueiredo et al. (2007)

Azospirillum brasilense, *Bacillus pantothenticus*, *Pseudomonas pieketti*	Rice (*Oryza sativa*)	Micro-plots	increased rice grain yield maximum upto 76.9%	Thakuria et al. (2004)
Pseudomonas spp., *Variovorax* spp., *Agrobacterium* spp., *Phyllobacterium* spp.	Canola (*Brassica napus*)	Growth chamber	significantly increased root dry weight ranging from 11 to 52%	Bertrand et al. (2001)
Bacillus spp.	Barley (*Hordeum vulgare*)	Greenhouse	increased root weight upto 16.7% and shoot weight upto 347%	Canbolat et al. (2006)
Bacillus subtilis	*Solanum lycopersicum* L. (tomato), *Abelmoschus esculentus* (okra), *Amaranthus* sp. (African spinach)	Greenhouse	dry biomass increased 31% for tomato, 36% for okra, and 83% for African spinach	Adesemoye et al. (2008)
Pseudomonas aeruginosa	*Solanum lycopersicum* L. (tomato), *Abelmoschus esculentus* (okra), *Amaranthus* sp. (African spinach)	Greenhouse	dry biomass increased 31% for tomato, 29% for okra, and 40% for African spinach	Adesemoye et al. (2008)
Unidentified PGPR isolate	wheat	gnotobiotic conditions	increases in root elongation (up to 17.3%), root dry weight (up to 13.5%), shoot elongation (up to 37.7%) and shoot dry weight (up to 36.3%) in inoculated wheat seedlings	Khalid et al. (2004)
Bradyrhizobium sp. (vigna) RM8	Greengram	pots	enhanced the nodule numbers by 82%, leghaemoglobin by 120%, seed yield by 34%, grain protein by 13%, root N by 41% and shoot N by 37% at 290 mg Ni/kg soil.	Wani et al. (2007a)
Mesorhizobium sp. RC3	Chickpea	pots	Increased the dry matter accumulation, number of nodules, seed yield and grain protein by 71, 86, 36 and 16%, respectively, compared to noninoculated plants. Nitrogen in roots and shoots increased by 46 and 40%, respectively, at 136 mg Cr/ kg.	Wani et al. (2008)
Rhizobium sp. RP5	Pea	pots	enhanced the dry matter, nodule numbers, root N, shoot N, leghaemoglobin, seed yield, and grain protein by 19%, 23%, 26%, 47%, 112%, 26%, and 8%, respectively, at 290 mg Ni/kg	Wani et al. (2007b)

2.2.3.1.2. Phytohormones

Another direct mechanism by which PGPR facilitates plant growth is the production of plant growth regulators or phytohormones (Azcón et al., 1978, Glick, 1995, Wani et al., 2007a, Wani et al., 2008). Frankenberger and Arshad, (1995) have discussed in detail the role of auxins, cytokinins, gibberellins, ethylene and abscisic acids (ABA) which, when applied to plants, have shown a substantial increase in growth and yield of plants. The production of phytohormones such as, auxins (IAA), cytokinins and gibberellins by natural soil microbial communities have been reported by various workers over the last 20 years (Giordano et al., 1999a, Giordano et al., 1999b, Poonguzhali et al., 2008, Singh et al., 2008, Selvakumar et al., 2008, Rajkumar and Freitas, 2008).

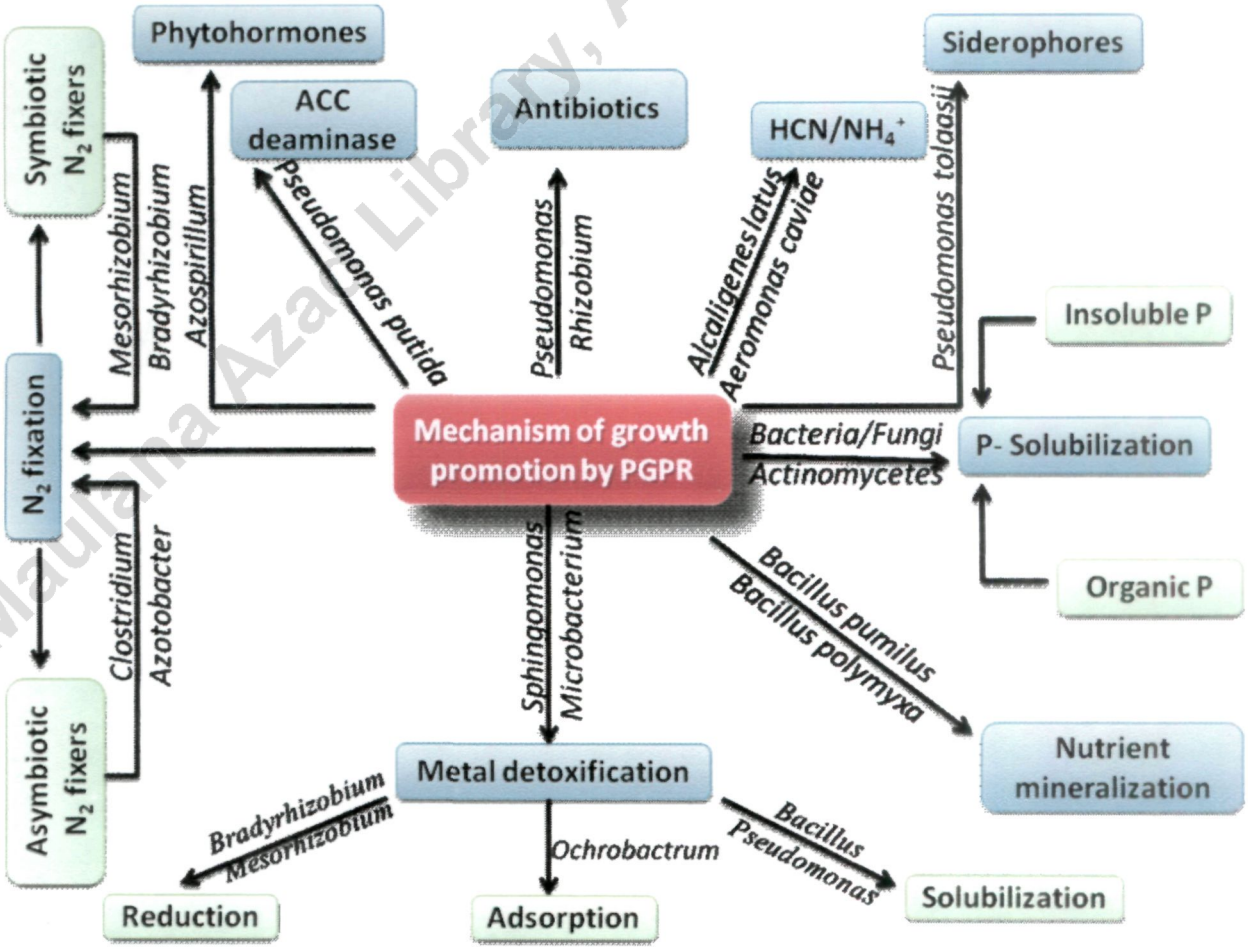


Fig. 2: A general scheme showing how PGPR promote plant growth

As an example, the production of indole-3-acetic acid (IAA) by microorganisms in the presence of the precursor tryptophan or peptone has been reported (Wani et al., 2008, Ahmad et al., 2008). Indole-3-acetic acid, a main auxin in plants is known to control many important physiological processes (Fig. 3). In plant cells, IAA is largely formed by *de novo* synthesis from tryptophan, which undergoes either oxidative deamination (via the formation of indole-3-pyruvic acid) or decarboxylation (via the formation of tryptamine, with indole-3-acetic aldehyde as an intermediate.

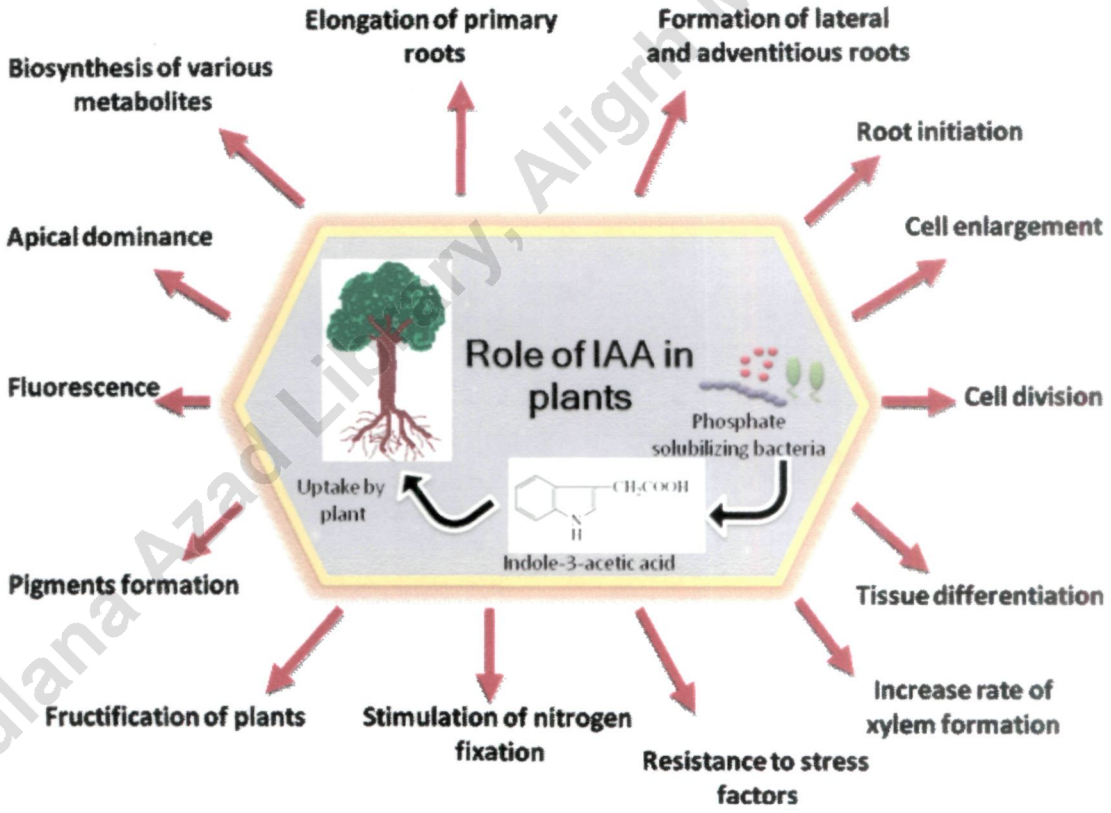


Fig. 3: Indole acetic acid affecting various stages of plant development

The synthesis of IAA by microbes involves one of the three pathways as presented in Fig. 4 (Patten and Glick, 1996): (1) IAA formation via indole-3-pyruvic acid and indole-3-acetic aldehyde is found in the majority of bacteria like, *Erwinia herbicola*; saprophytic species of the genera *Agrobacterium* and *Pseudomonas*; certain representatives of *Bradyrhizobium, Rhizobium, Azospirillum, Klebsiella*, and *Enterobacter*, (2) The conversion of tryptophan into indole-3-acetic aldehyde may involve an alternative pathway in which tryptamine is formed. This pathway is believed to operate in pseudomonads and azospirilla and (3) IAA biosynthesis via indole-3-

acetamide formation is reported for phytopathogenic bacteria *Agrobacterium tumefaciens*, *Pseudomonas syringae*, and *E. herbicola*; saprophytic pseudomonads like (e.g. *Pseudomonas putida* and *P. fluorescens*). The genes controlling IAA synthesis via this pathway are also reported in symbiotic bacteria (e.g. *Rhizobium* spp., *Bradyrhizobium* spp., and *Azospirillum* spp.), although the activity of the corresponding enzymes is either negligible or not detectable. (4) IAA biosynthesis that involves tryptophan conversion into indole-3-acetonitrile is found in plants, *Alcaligenes faecalis*, and possibly the cyanobacterium (*Synechocystis* sp.) and (5) The tryptophan-independent pathway, more common in plants, is also found in microorganisms (azospirilla and cyanobacteria). However, the synthesis of IAA using this pathway is reported to be insignificant, and the mechanisms are largely unknown(Fig.4).

Many bacteria are known to synthesize auxins using such pathways and help the plants to grow better. In this context, rhizospheric microflora colonizing plant roots are of paramount importance in the conversion of tryptophan into IAA. And hence, the removal of tryptophan from the culture medium leads to decrease in the level of IAA by the microorganisms. However, the amount of the auxins formed depends primarily on the composition of the medium and the conditions (e.g., temperature, aeration, etc.) of bacterial growth. Bacteria in general, forms maximum amount of IAA during the steady-state stage of their growth while ammonium ions and glutamine inhibit IAA biosynthesis (Tsavkelova et al., 2006). The genes involved in IAA synthesis in bacterial strains may be plasmid or chromosomal borne. For example, pathogenic bacteria contain Ti plasmids that control the formation of the phytohormone, whereas in saprophytic microorganisms, auxin biosynthesis is governed by chromosomal genes (Tsavkelova et al., 2006).

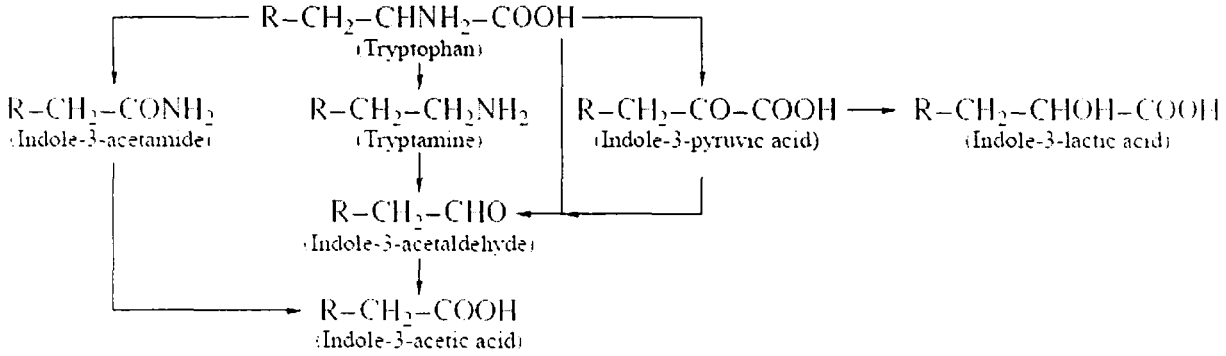


Fig. 4: Biosynthetic pathways of IAA synthesis in bacteria

It is reported that 80% of microorganisms isolated from the rhizosphere of various crops possess the ability to synthesize and release auxins as secondary metabolites (Loper and Schroth, 1986). Of the various PGPR strains, bacteria belonging to the genera *Azospirillum*, *Pseudomonas*, *Xanthomonas*, and *Rhizobium* as well as *Alcaligenes*, *Enterobacter*, *Acetobacter* and *Bradyrhizobium* have been shown to produce auxins which help in stimulating plant growth (Egamberdieva et al., 2007, Wani et al., 2007a, Poonguzhali et al., 2008, Kumar et al., 2008). However, the extent of IAA production by bacterial strains could be different due in part to the involvement of biosynthetic pathways, location of the genes, regulatory sequences, and the presence of enzymes to convert active free IAA into conjugated forms. Moreover, the synthesis of IAA is also influenced by environmental factors (Patten and Glick, 1996). Synthesis of IAA by *Rhizobium* spp. in the presence and absence of tryptophan has also been demonstrated (Wani et al., 2007b). Bent et al. (2001) reported that the concentration of indole compounds by three different strains, *Paenibacillus polymyxa* (L6), *P. poly*myxa (Pw-2), and *Pseudomonas fluorescens* (M20) increased with increasing rate of tryptophan (0-200 mg/ml) at different incubation interval.

2.2.3.1.3. Microbial phosphate solubilization

Phosphorus (P), a major plant nutrient is required for various metabolic processes such as, energy transfer, signal transduction, macro-molecular biosynthesis, photosynthesis and respiration (Khan et al., 2009a). Phosphorus however, is also one of the major nutrients limiting plant growth (Fernandez et al., 2007). Worldwide, 5.7 billion hectares land contain too little available P for sustaining optimal crop production (Hinsinger, 2001). Phosphorus ion concentration in most soils ranges from 0.1 to 10 μM; P required for optimal growth ranges from 1 to 5 μM for grasses and 5 to 60 μM for high demanding crops such as tomato (*Lycopersicum esculentum*) and pea (*Pisum sativum*) (Raghothama, 1999). Sub-optimal levels of P, can however, lead to a 5 to 15% losses in the yield of crops (Hinsinger, 2001). Phosphorus is present in the soils both in organic and inorganic forms (Fig. 5). Of which, organic forms, as found in humus and other organic materials including decayed plant, animal and microbial tissues, is an important reservoir of immobilized P accounting for about 20–80% of total soil P (Richardson, 1994). Phosphorus in labile organic compounds can be slowly mineralized as available inorganic

P or it can be immobilized as part of the soil organic matter (Mckenzie and Roberts, 1990). The process of mineralization or immobilization is carried out by microorganisms and is highly influenced by soil moisture and temperature. Mineralization and immobilization are most rapid in warm, well-drained soils (Busman et al., 2002). Soil inorganic P is however, controlled mainly by solution pH and the concentration of cations and in most soils, maximum P availability occurs between pH 5.5 to 7. Within this pH range, P is fixed by hydrous oxides of Fe, Al, and Mn while between pH 6 to 8 and pH 6.5 to 8.5, P is fixed by silicate minerals and Ca, respectively. As a consequence, the most efficient use of P in neutral and calcareous soils occurs between pH 6 to 7.

However, the majority of P is unavailable for uptake by plants due to its rapid rate of fixation/complex formation with other elements of soils. Therefore, phosphatic fertilizers are applied to soil to replenish the P demands of growing plants. However, a large portion of soluble inorganic P applied to the soil as fertilizer is immobilized rapidly and becomes unavailable to plants (Goldstein, 1986). For instance, in the United States, an average 29% of P added in fertilizer and manure is removed by harvesting crops (Sharpley, 2006). Moreover, the concentration of soluble P in soil solution is very low (400–1,200 mg kg^{-1} of soil) (Ehrlich, 1990, Rodriguez and Fraga, 1999). Attempts to overcome the P deficiency by applying phosphatic fertilizers is expensive and ecologically unsafe practice because the efficiency of the added P fertilizer is as low as about 10% (Werft and Dekkers, 1996). This has led to search environment-friendly and economically feasible alternative strategies for improving crop production in low P soils. In this context, organisms endowed with phosphate solubilizing activity, often termed phosphate solubilizing microorganisms (PSM), may provide the available forms of P to the plants and hence a viable substitute to chemical phosphatic fertilizers (Khan et al., 2006c). Of the various PSM (s) inhabiting rhizosphere, phosphate-solubilizing bacteria (PSB) are considered as promising biofertilizers since they can supply plants with P from sources otherwise poorly available (Khan et al., 2006c). Though, PSB are commonly found in most soils (Venkateswarlu et al., 1984, Kucey et al., 1989, Das et al., 2003c, Peix et al., 2003, Peix et al., 2004, Wani et al., 2007c); their establishment and performances are severely affected by environmental factors especially under stress conditions (Wani et al., 2007c, Tilak, 1991). However, the beneficial effects of the inoculation with PSB, used either alone (Poonguzhali et

al., 2008, Chen et al., 2008, Singh et al., 2008) or in combination with other rhizospheric microbes have been reported (Zaidi et al., 2003, Zaidi and Khan, 2005, Zaidi and Khan, 2006, Vikram and Hamzehzarghani, 2008).

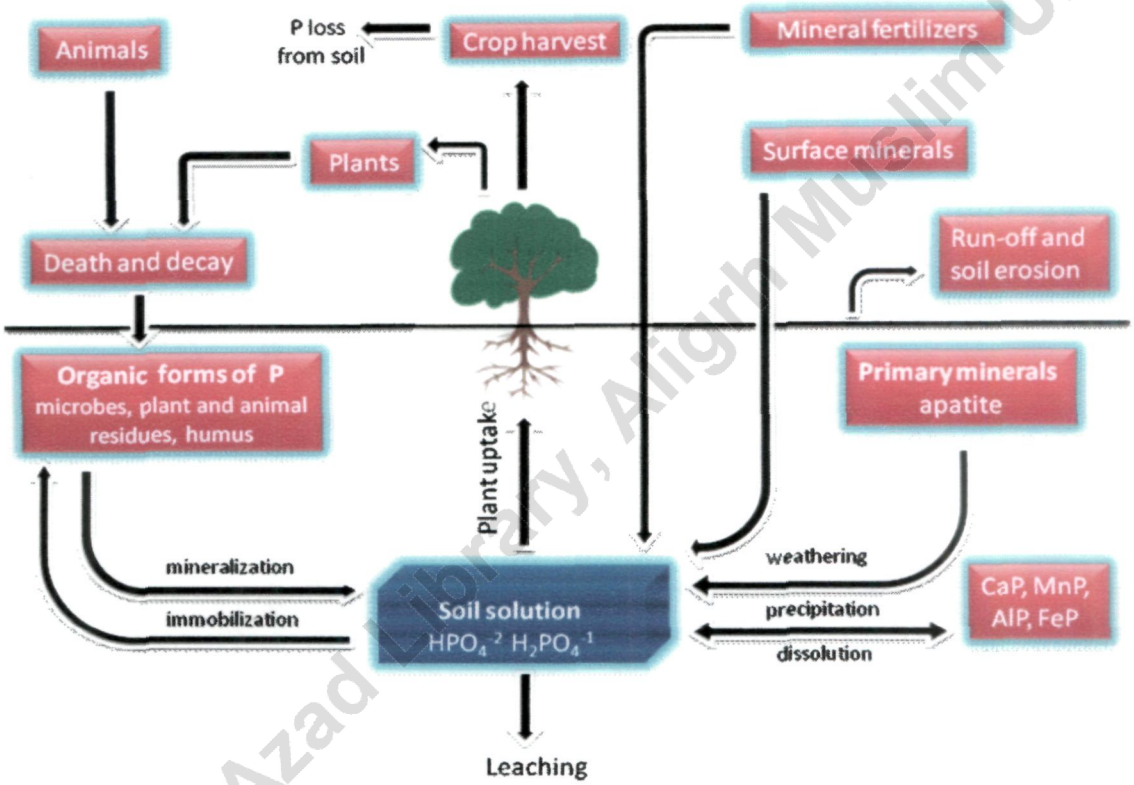


Fig. 5: Movement of phosphorus in soils

Besides providing P to the plants, the PS bacteria also augment the growth of plants by stimulating the efficiency of BNF, enhancing the availability of other trace elements (such as iron, zinc) and by synthesizing important plant growth promoting substances (Ponmurugan and Gopi, 2006, Wani et al. 2007b, Mittal et al., 2008) including siderophores (Vassilev et al., 2006, Wani et al., 2008), antibiotics (Dilantha et al., 2006) and providing protection to plants against soil borne pathogens (biocontrol) (Khan et al., 2002, Singh et al., 2008). Accordingly, these bacterial communities when used either singly (Chen et al., 2008) or as consortia, in combination with other rhizosphere microbes (Wani et al., 2007a; Wani et al 2007b, Mishra et al., 2009) have shown a substantial improvement in crop productivity in different agro-ecosystems. In the following section, an attempt is made to identify such natural PS bacteria and the mechanisms through which they solubilize the insoluble P in soils.

2.2.3.1.3.1. Mechanism of P solubilization

Several theories have been proposed for the solubilization of insoluble P. These include- (i) the sink theory (Halvorson et al., 1990) (ii) the organic acid theory (Cunningham and Kuiack, 1992) and (ii) the acidification by H^+ excretion theory (Illmer and Schinner, 1995). The sink theory involves the indirect dissolution of Ca-P compounds by continuous removal of P from broth. The sink theory, however, can be used to explain mineralization of organic P compounds in which the P content in biomass of organisms is consistently correlated with the decomposition of P-containing organic substrates (Dighton and Boddy, 1989). In comparison, the organic acid theory is well recognized and most widely accepted mechanism of P solubilization by PS bacteria. These organisms are able to solubilize 'unavailable' forms of P through their metabolic activities, by excreting organic acids which chelate mineral ions or drop the pH to bring P into solution (Cunningham and Kuiack, 1992). The organic acid produced by bacteria (Table 3) in turn, leads to acidification of microbial cells and their surroundings and, consequently, the release of P ions from the P mineral by H^+ substitution for Ca^{2+} (Goldstein, 1994). Similar evidence on the involvement of organic acids in the solubilization of insoluble P is reported by others (Maliha et al., 2004, Pradhan and Sukla, 2005, Ponmurugan and Gopi 2006). However, a lot of P was fixed in acidic soil (such as red soil) accumulating Fe or Al ions and no correlation was found between pH and the amount of solubilized P (Asea et al. 1988). It is still unexplained that why no substantial amounts of organic acid production could be detected from some PS microorganisms (Illmer and Schinner 1992, Chen et al. 2006). For this reason, alternative possibilities other than organic acids for insoluble inorganic P solubilization have been proposed, such as the release of H+ (Illmer and Schinner 1995), the production of chelating substances and inorganic acids (Khan et al., 2006c, Khan et al., 2009a). The H^+ release is thought to be associated with cation assimilation, such as ammonium ion (NH_4^+). Hydrogen ion ($H^{+)}$ excretion accompanying NH_4^+ assimilation is responsible for P solubilization. The excreted H^+ accompanying the decrease in pH acted as a solvent agent for P solubilization. The NH_4^+-N had the lowest pH value among different N sources and was the most effective for P solubilization in liquid cultures by *P. bilaiae* (Cunningham and Kuiack, 1992). Recently, Yi et al. (2008) used four bacterial strains of *Enterobacter* sp. (EnHy-401), *Arthrobacter* sp. (ArHy-505), *Azotobacter* sp. (AzHy-510) and *Enterobacter* sp. (EnHy-402), which had the ability to

solubilize tri-calcium phosphate (TCP), in order to study the mechanism of P-solubilization. These PS bacteria produced a significant amount of exopolysaccharides (EPS) and demonstrated a stronger ability for P-solubilization. Of these, the strain EnHy-401 with the highest EPS and organic acids production had a stronger capacity for P solubilization than the others. Further studies demonstrated that addition of EPS into medium could increase the amount of P solubilized by organic acid, but failed to release P from TCP alone. However, certain enzymes (e.g., acid phosphatases) play a major role in the mineralization of organic P in soil (Rodriguez and Fraga, 1999). Different mechanisms involved in solubilization of phosphate are shown in Fig. 6.

Table 3: Organic acids involved in P solubilization and produced by phosphate solubilizing bacteria

Phosphate solubilizing bacterial communities	Organic acids	References
Bacillus, Rhodococcus, Arthrobacter, Serratia, Chryseobacterium, Delftia, Gordonia, Phyllobacterium, Arthrobacter ureafaciens, Phyllobacterium myrsinacearum, Rhodococcus erythropolis, Delftia sp.	Citric acid, gluconic acid, lactic acid, succinic acid, propionic acid	Chen et al. (2006)
Enterobacter intermedium	2-ketogluconic	Hoon et al. (2003)
Bacillus amyloliquefaciens, B. licheniformis, B. atrophaeus, Penibacillus macerans, Vibrio proteolyticus, xanthobacter agilis, Enterobacter aerogenes, E. taylorae, E. asburiae, Kluyvera cryocrescens, Pseudomonas aerogenes, Chryseomonas luteola	Lactic acid, itaconic acid, isovaleric acid, isobutyric acid, acetic acid	Vazquez et al. (2000)
Bacillus polymyxa, B. licheniformis, Bacillus spp.	Oxalic acid, citric acid	Gupta et al. (1994)
Pseudomonas striata	gluconic acid, tartaric acid, citric acid, maleic acid, succinic acid, glyoxalic acid	Vikram et al. (2007)
Arthrobacter sp.	Oxalic acid, malonic acid	Banik and Dey (1982)
Bacillus firmus	2-ketogluconic acid, succinic acid	Banik and Dey (1982)

2.2.3.1.3.2. Agronomic utility of P solubilizing bacteria

Inoculation of phosphate solubilizing microorganism (PSM) when used alone or as mixtures in soils, has also been shown to improve solubilization of insoluble P resulting in higher crop yields (. Peix et al., 2001, Sundara et al. 2002, Wani et al., 2007c, Shaharoona et al., 2008). Such PS bacterial strains demonstrated a substantial increase in plant growth (Katiyar and Goel, 2003, Zaidi and Khan 2006, Wani et. al., 2007c) and hence, PSM(s) could be of tremendous importance to the development of plants in different agro-ecolological niches.

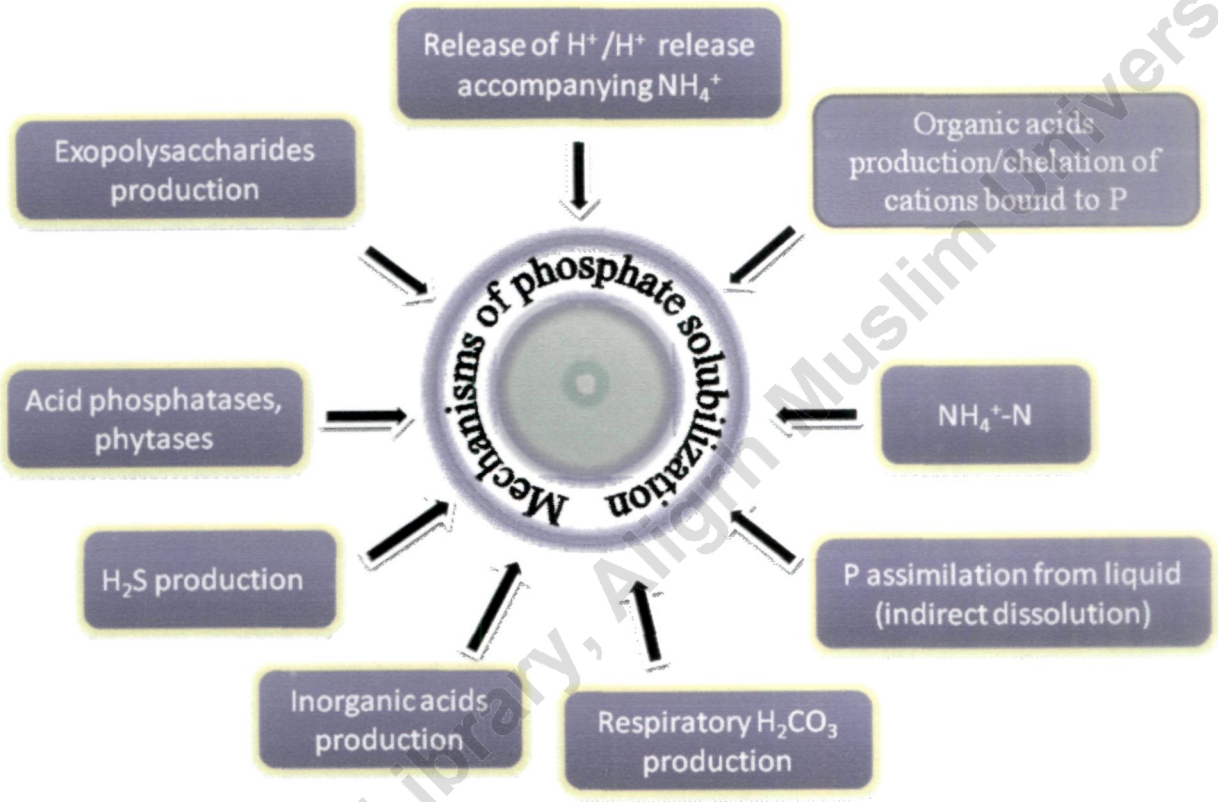


Fig. 6: Mechanisms of P solubilization by phosphate solubilizing bacteria

The PS bacteria promote plant growth by synthesizing various compounds (Table 4). In the following section, an attempt is made to evaluate the impact of PS bacteria, when used either singly or in synergism with other plant growth promoting rhizobacteria, on the performance of crops grown in different agro-ecosystems. The inoculation of PS bacteria is a promising agrobio-technique because it increases the availability of P in soils.

The inoculation of a good solubilizer of Fe and P, *Sinorhizobium meliloti 3DOh13*, used for alfalfa (*Medicago sativa*), *Bradyrhizobium japonicum TIIIB* used for soybean (*Glycine max*) and two PS strains of *Pseudomonas putida* (*SP21* and *SP22*) used for both legumes has shown a significant effect on these crops (Susana et al., 2006). Similarly, the soybean plants inoculated with *Burkholderia* sp. (PER2F) had the highest aerial height and showed an appropriate N/P ratio but inoculation with *Enterobacter* sp., and *Bradyrhizobium* sp. did not increase P uptake by plants (Fernández et al. 2007). They suggested that PS bacterial inoculation does not necessarily improve P nutrition in soybean, nor was there any relationship between P availability in the soil plate assay and P content in the shoot of soybean grown in greenhouse.

Table 4 Growth promoting substances released by phosphate solubilizing bacteria

Phosphate solubilizing bacteria	Plant growth promoting traits	References
Pseudomonas sp.	ACC deaminase, IAA, siderophore	Poonguzhali et al. (2008)
Bacillus subtilis	IAA, siderophore, antifungal activity	Singh et al. 2008
Serratia marcescens	IAA, siderophore, HCN	Selvakumar et al. (2008)
Pseudomonas fluorescens	ACC deaminase	Shaharoona et al. (2008)
Acinetobacter sp., *Pseudomonas* sp.	ACC deaminase, IAA, antifungal activity, N_2- fixation	Indiragandhi et al. (2008)
Enterobacter sp.	ACC deaminase, IAA, siderophore	Kumar et al. (2008)
Burkholderia	ACC deaminase, IAA, siderophore, heavy metal solubilization	Jiang et al. (2008)
Pseudomonas jessenii	ACC deaminase, IAA, siderophore, heavy metal solubilization	Rajkumar and Freitas, (2008)
Pseudomonas aeruginosa	ACC deaminase, IAA, siderophore	Ganesan (2008)
Pseudomonas sp.	ACC deaminase, IAA, siderophore, heavy metal solubilization	Rajkumar and Freitas (2008)
Azotobacter sp., *Mesorhizobium* sp., *Pseudomonas* sp., *Bacillus* sp.	IAA, siderophore, antifungal activity, ammonia production, HCN	Ahmad et al. (2008)
Bacillus spp.	IAA, siderophores, ammonia production, HCN, chromium reduction, metal solubilization	Wani et al. (2007a, b, c)
Bacillus subtilis	IAA	Zaidi et al. (2006)
Pseudomonas sp., *Bacillus* sp.	IAA, siderophore	Rajkumar et al. (2006)
Pseudomonas putida	Antifungal activity, siderophore, HCN	Pandey et al. (2006)
Pseudomonas fluorescens PRS_9, *Pseudomonas fluorescens* GRS_1	IAA, siderophores	Gupta et al. (2005)

From this study, it was concluded that the selection of efficient PS strains as possible inoculation tools for P-deficient soils should focus on the results obtained in soil plate assays, greenhouse experiments, and field trials. Furthermore, a PS strain of *Mesorhizobium mediterraneum* (PECA21) substantially increased the growth and P content in chickpea plants when grown in soil amended with or without phosphates (TCP) in a growth chamber experiment (Peix et al., 2001). The strain PECA21 could mobilize P efficiently and increased the P contents by 100%. Also, the dry matter accumulation, N, K, Ca and Mg content were dramatically increased in inoculated plants, grown in soil treated with insoluble P. These results therefore, suggested that the inoculation of soil with rhizobia should not be considered only for its N_2 fixing potential, but also for its ability to solubilize P. Likewise, inoculation of greengram seeds with PS bacteria revealed a highest nodule number, nodule dry weight, shoot dry matter and total dry matter, P-content and P uptake compared to RP and single super phosphate (SSP) control. However, plant growth promoting ability of microbial communities varied considerably (Vikram and Hamzehzarghani, 2008).

Recently, some instances have also been reported where plant growth was markedly enhanced using consortia of PS bacteria and other growth promoting organisms. For examples, N_2 fixers and PS bacteria when inoculated together increased N and P contents in legumes (Gull et al., 2004; Wani et al., 2007a, Wani et al., 2007b). The N_2 fixing organisms besides providing N to the plants could also enhance N status of soil, alone or in combination with PS bacteria. For instance, the inoculation of PS bacterium *Pseudomonas striata* and N_2 fixing bacterium [*Rhizobium* sp. (vigna)] substantially increased the yield of greengram (Khan et al., 1997) and chickpea (Wani et al., 2007a, Wani et al., 2007b) compared to single inoculation of either PS bacterium or N_2 fixer. Similarly, the combined application of PS bacteria and N_2 fixers significantly increased the overall performance of soybean (Dubey, 2001), cereals and other legume crops (Kumar et al., 2001, Whitelaw, 2000). When *Mesorhizobium ciceri* and PS *Pseudomonas* or *Bacillus* were used together in sandy clay loam soils increased nodulation, available P of soil as well as dry matter of the plants, grain yield and P and N uptake by the plants (Wani et al., 2007a). In a pot experiment, lentil seeds were inoculated with *Rhizobium leguminosarum* along with increasing doses (50, 100, 200, 400 kg / feddan, 1 feddan = 0.42ha) of RP with or without a 1: 1 mixture of elemental S and RP in the presence or absence of PS bacteria. Plant dry weight and N, P, Fe, Zn, Mn and Cu uptake increased with RP, S and PS bacteria compared with untreated control. Dry matter yield and nutrient uptake was slightly higher with S application (Saber and Kabesh, 1990). A significant increase in mungbean yield was observed with the inoculation of *Bradyrhizobium* spp. and PS bacteria along with P fertilizers (Khan et al., 1997). Moreover, the microbes that are involved in P solubilization as well as better scavenging of soluble P can enhance plant growth by improving the efficiency of BNF, accelerating the availability of other trace elements and by production of phytohormones. Accordingly, increase in yield of various legumes have been observed following seed or soil inoculation with N_2 fixing organisms and PS bacteria (Wani et al., 2007b) or PS bacteria (s) and AM fungus (Zaidi and Khan 2006, Khan and Zaidi, 2007). It has further been suggested that about 50% of P fertilizer requirement could be saved by the combined inoculation of *Rhizobium* with *Bacillus* in groundnut (Natarajan and Subramanian, 1995).

Gull et al. (2004) reported that chickpea growth, shoot phosphorus and nitrogen concentrations, nodulation efficiency and nitrogenase activity were significantly enhanced in

presence phosphate solubilizing bacterial strains isolated from rhizosphere, roots and nodules of chickpea. Phosphate solubilizing strains, CPS-2, CPS-3 and Ca-18 had the maximum positive effect on shoot length, shoot dry weight and nodulation of chickpea plants. Valverde et al. (2006) tested the effect of single and dual inoculations of *Mesorhizobium ciceri* C-2/2 and PS bacterial strain *Pseudomonas jessenii* PS06 on chickpea growth. They observed that under greenhouse conditions, plants inoculated with *Mesorhizobium ciceri* alone had the highest shoot dry weight and that with *P. jessenii* yielded a shoot dry weight 14% greater than the uninoculated control treatment. However, the co-inoculation of C-2/2 with PS06 resulted in a decrease in shoot dry weight with respect to the inoculation with C-2/2 alone. Under field conditions, plants inoculated with *M. ciceri* C-2/2, in single or dual inoculation, produced higher nodule fresh weight, nodule number and shoot N content than the other treatments. Inoculation with *P. jessenii* PS06 had no significant effect on plant growth. However, the co-inoculation treatment ranked the highest in seed yield (52% greater than the uninoculated control treatment) and nodule fresh weight. This study suggested that phosphate solubilizing bacteria can act synergistically with N_2 fixer in promoting chickpea growth.

The rhizospheric microorganisms possessing PS activity are also endowed with intrinsic ability to reduce the toxicity of certain agrochemicals in contaminated soils (Wani et al., 2007c). As a result of these properties, PS bacteria when applied to seeds or incorporated into the soil, reduce the hazardous effects of such pollutants (Wani et al., 2007b) and protect the plants against the toxic effects of such chemicals and consequently enhance the growth and yield of plants (Rajkumar et al. 2006). Therefore, the use of such PS bacteria for reduction/detoxification of agrochemicals is one of the preferred choices and is considered as cost effective approach in bioremediation technologies. As an example, Bano and Musarrat (2003) isolated phorate degrading bacteria belonging to the genera *Rhizobium* strain PS1, *Pseudomonas* strain PS2 and *Proteous* strain PS3 from agricultural soils. These bacteria exhibited substantial phosphate solubilization, synthesize indole acetic acid, and produced siderophores. The strain PS-3 also showed anti-fungal activity against a phytopathogen *Fusarium oxysporum*. As a result of the multifarious biological properties, the strains are considered important bioresource for developing efficient bioinoculant.

2.2.3.1.4. Siderophores

Iron-chelating molecules termed siderophores are generally less than 1000 molecular weight and are produced by many microorganisms. More than 500 different siderophores, mostly produced by Gram-positive and Gram-negative bacteria, have been described. Despite this great variety, most have a peptide backbone with several non-protein amino acid analogs including both modified and D-amino acids. Various types of siderophores are presented in Fig. 7. In general, rhizosphere bacteria differs with respect to siderophore cross-utilizing abilities; some are proficient at using siderophores of the same genus (homologous siderophores) while others could utilize siderophores produced by other rhizospheric bacteria of different genera (heterologous siderophores) (Joshi et al., 2006).

Siderophore production and utilization by rhizobacteria is of particular interest due to the dominant role of iron in the nitrogen fixation and assimilation process. The iron enzymes involved include nitrogenase, leghemoglobin, ferredoxin and hydrogenase with nitrogenase and leghemoglobin constituting up to 12% and 30% of total protein in the bacterial and infected plant cells, respectively (Verma and Long, 1983).

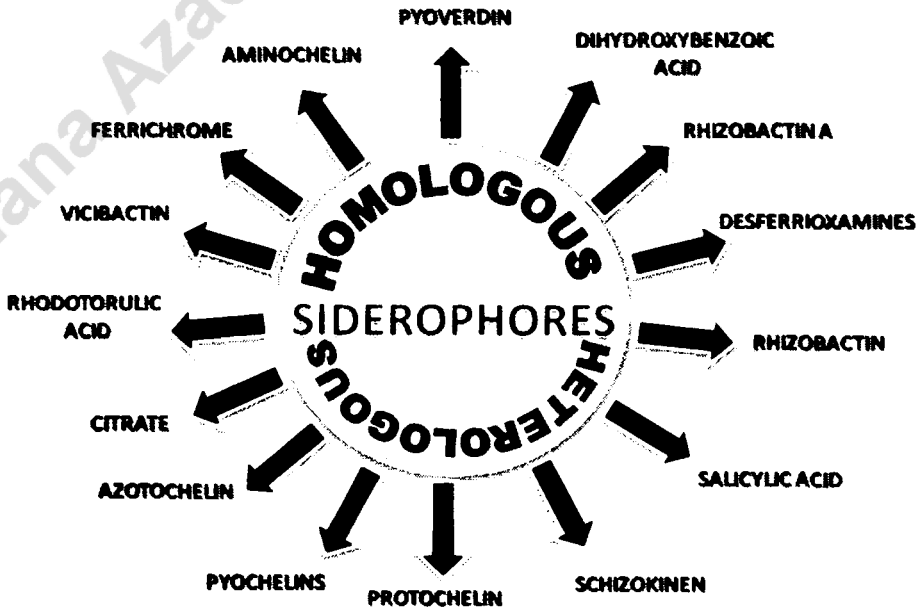


Fig. 7: Different types of homologous and hetrologous siderophores

Siderophores chelate iron with high affinity (the calculated affinity constant being above 10^{30} M^{-1}). Siderophores are highly electronegative and bind Fe (III), preferentially forming a hexacoordinated complex. The iron ligation groups have been tentatively classified into three main chemical types: hydroxamate (e.g., aerobactin and ferrichrome), catechol rings (e.g., enterobactin) and hydroxyacid (e.g., pyochelin). Some siderophores contain more than one of these three iron-chelating groups.

One of the suggested modes of growth promotion of nodulated legumes under field conditions is by microbial production of siderophores, which facilitate the uptake of iron from the environment (Kloepper et al., 1980, Katiyar and Goel, 2004a, Katiyar and Goel, 2004b). The effects of microbial siderophores on growth and development of plants are presented in Fig. 8. A nodulated legume has been found to have an increased demand for iron compared to that of a non-nodulated plant (Deryto and Skorupska, 1993). For example, *Pseudomonas* sp. strain 267 enhanced symbiotic N_2 fixation in clover under gnotobitoic conditions, produced fluorescent siderophores under low-iron conditions and secreted B group vitamins (Kozaczuk and Skorupska, 2001).

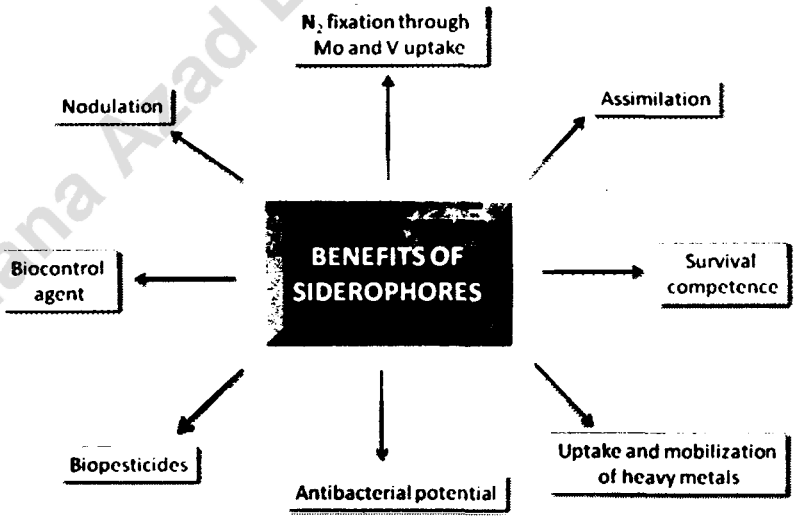


Fig. 8: Impact of microbially secreted siderophores on plant growth

However, Tn5 insertion mutants of strain 267 defective in siderophore production did not differ from the wild-type in promoting the growth of clover (*Medicago sativa*), suggesting that the siderophore production had no effect on stimulating nodulation. In contrast, Gill et al. (1991) demonstrated that mutants of *Rhizobium melioti* that were unable to produce siderophores were able to nodulate the plants, but the efficiency of N_2 fixation was less compared to the wild-type,

indicating the importance of iron in N_2 fixation. In a similar study, *Kluyvera ascorbata*, a siderophore-producing PGPR, was able to protect plants from heavy metal toxicity (Burd et al., 1998).

2.3. Toxicity of pesticides to soil microorganisms and plants

Soil microorganisms are an important and diverse community that catalyzes many processes important for soil fertility and plant growth. The processes may be the cycling of nutrients from soil and fertilizers, and the direct transfer of nutrients to crops by microbes including symbiotic rhizobia and other plant growth promoting rhizobacteria. However, such beneficial microbial communities of soils are greatly influenced by various factors including the agrochemicals (Fig. 9). In modern agricultural practices, agronomists are applying pesticides in order to augment the productivity of various crops. A pesticide is any substance or mixture of substances intended for preventing, destroying, repelling or mitigating any pest and has been classified as presented in Fig. 10. A microorganism can be tolerant or resistant (slightly or not affected) towards a pesticides. Pesticides act on sensitive species by interfering with vital metabolic process.

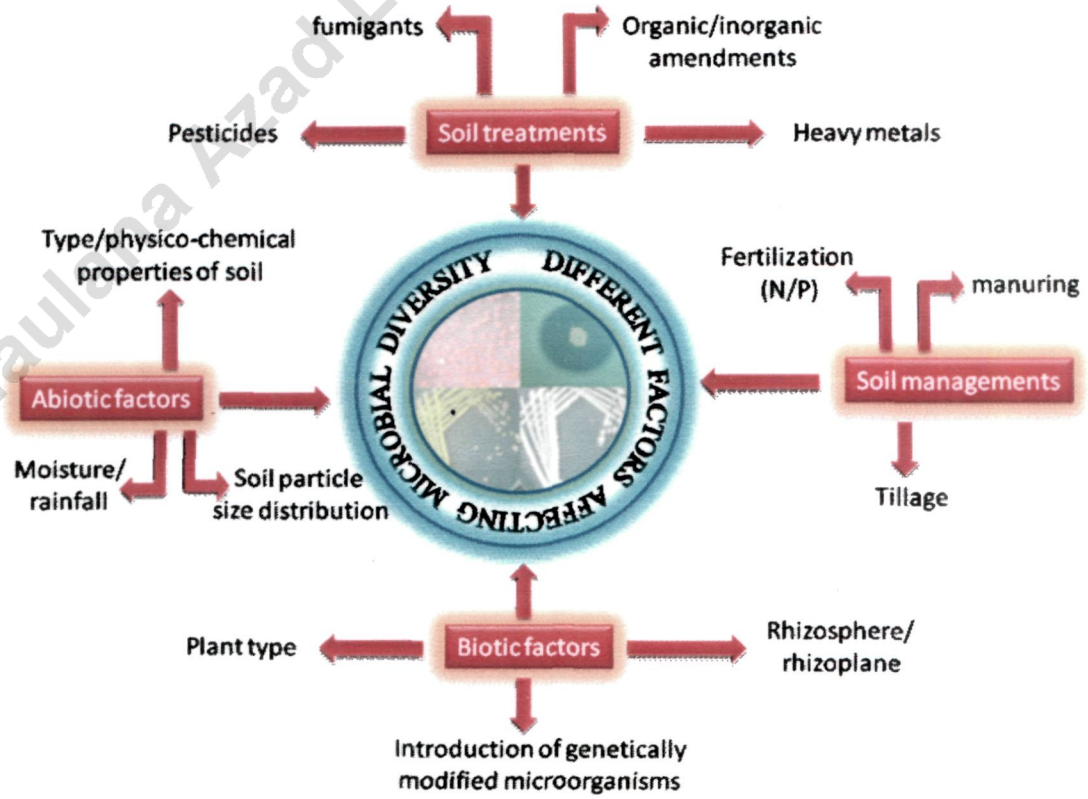


Fig. 9: Various factors affecting soil microbial communities

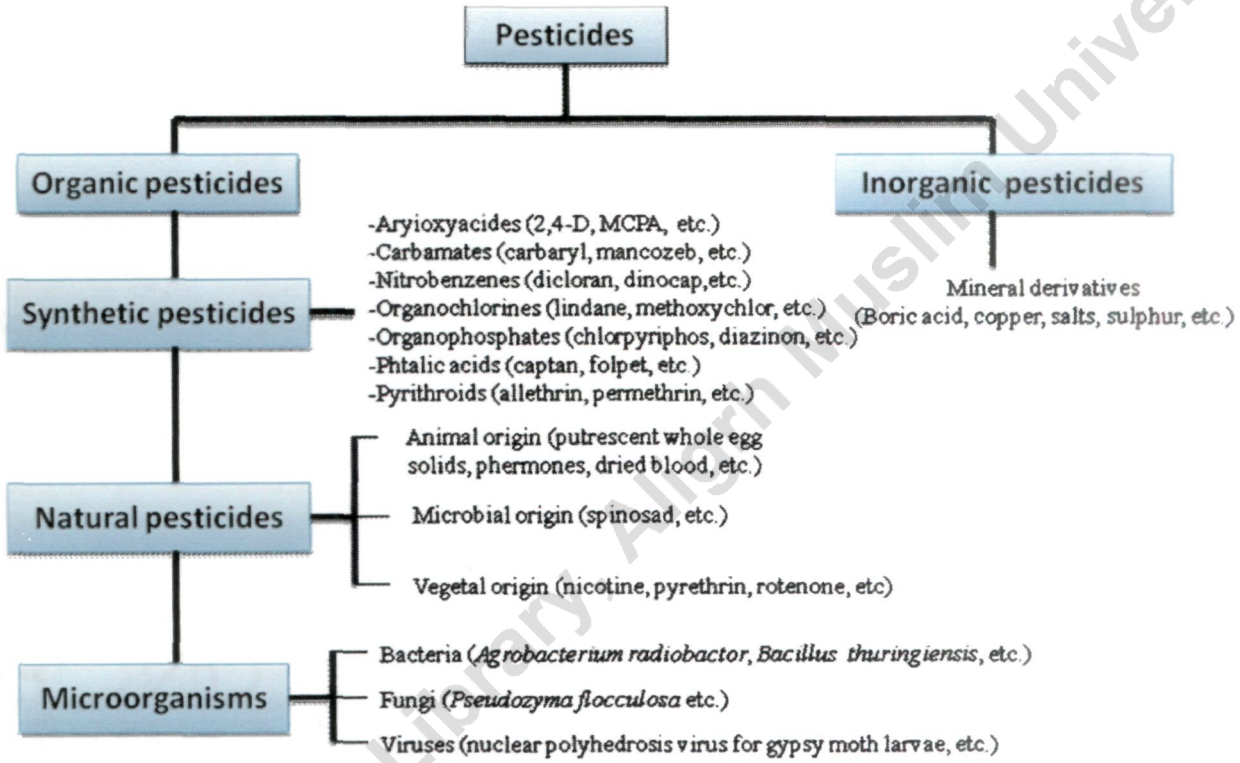


Fig. 10: General classification of pesticides

The consistent and injudicious use of synthetic pesticides has however, become a major threat to beneficial soil microbes (Zahran, 1999, Srinivas et al., 2008) and in turn affects the sustainability of agricultural crops. Globally, the greater concern is therefore, as to how to minimize or reduce the effects of pesticides so that the consequential impact of these chemicals on the microorganisms involved in nutrient cycling, vis-a-vis the productivity of crops could be saved. In the following section, an attempt is made to highlight the impact of herbicides, insecticides and fungicides on soil microflora and agronomic crops.

2.3.1 Herbicidal impact on rhizobacteria and crops

Herbicidal effect on soil microflora and their activities (Table 6) may be of practical significance because of the possible inhibition of the biological activities of microbes that subsequently lead to decreased soil fertility. For example, glyphosate (N-(phosphonomethyl)glycine, Roundup_R), a broad spectrum, nonselective herbicide used for post-emergent control of a wide range of weeds, is the most widely applied herbicide due to the

introduction and broad acceptance of genetically-modified (GM), glyphosate-resistant (GR) crop varieties in the late 1990's (Vencill, 2002). Glyphosate is systemic and not readily metabolized by plants; it is translocated and may accumulate in meristematic regions including roots and nodules (Reddy et al., 2000, Means et al., 2007). In this context, glyphosate when applied at a rate of 2.16 mg/kg soil increased microbial activity (increase of 10–15 % in CO_2 evolution and a 9-19 % in FDA hydrolysis) over a period of 32 days compared with the same type of soil which never received glyphosate (Araujo et al., 2003).

The changes in aerobic bacteria, autotrophic nitrifiers, respiration and nitrification in soils treated with cinosulfuron at 42 (field rate) and 4200 µg/kg occured one and four weeks after incubation under laboratory conditions. Of the measured parameters, only nitrification was slightly inhibited by the cinosulfuron treatment, even at the field rate. While, the higher rates of cinosulfuron (100 mg/l) negatively affected the growth of aerobic bacteria, fungi and *Azotobacter* strains under conditions similar to those of soil environment (Allievi and Gigliotti, 2001). Similarly, trifluralin at lower concentrations (from 0.5 mg/mg dry soil to lower than 10 mg/mg dry soil) stimulated the growth of soil bacteria, and the pure cultures of *Azotobacter chroococcum* and *Bradyrhizobium japonicum*. Interestingly, Not only the populations of the two species of nitrogen fixing bacteria increased at lower concentrations of trifluralin, but also the size of colonies enlarged and appeared very quickly. In comparision, higher concentrations of trifluralin restricted the formation of microbial colonies and the acetylene reduction activity (ARA) of *A. chroococcum* suggesting that the microbial communities of soils could utilize trifluralin as sole C and N sources for their growth (Hang et al., 2001). When applied at lower rate, the commonly used herbicides in agronomic practices (atrazine and metolachlor) have been shown to affect soil pH and percentage organic matter. However, changes in the same parameters were significant only for atrazine treated soils. Both of these herbicides at the tested rates resulted in decrease in microbial counts and consequently eliminated some of the microbial species. Among bacteria, species of *Pseudomonas* and *Bacillus* were the most common microorganisms isolated from herbicide treated soils (Ayasina and Oso, 2006).

In a study, application of pendimethalin, prometryn, and trifluralin herbicides at the rates of 1, 2, and 4 µg a.i./g soil caused significant reductions in populations of most of the isolates in

the rhizosphere, 14 days after the release of *Pseudomonas fluorescens* and *Burkholderia cepacia* isolates into the soil by seed coating. However, the response of the isolates to the herbicides varied considerably and influenced by the isolate and the type and concentration of the herbicides. The impact of pendimethalin, prometryn, and trifluralin at 2.4, 3.6, and 1.8 µg a.i./g soils respectively, on the population of *Burkholderia cepacia* in cotton (*Gossypium* sp.) rhizosphere at one site declined with time during a four week period of monitoring following the release of the isolate into the soil by seed coating. Moreover, pendimethalin and prometryn caused significant reduction in the population of *Burkholderia cepacia* in cotton rhizosphere 15 days after sowing (Heydari et al., 1997). Simazine added to culture media at 10, 50 or 100 µg/ml affected the quantitative production of B-group vitamins (thiamin, niacin, pantothenic acid, cyanocobalamin and biotin) by *Azotobacter vinelandii* strain ATCC 12837 and *A. chroococcum* strain H23. The responses however differed among strains and were influenced by growth conditions. Effect of the herbicide on vitamin production was more pronounced when the strains were grown in dialysed-soil medium, similar to the natural habitat of the organisms (Murcia et al., 1997). Atrazine, terbuthylazine, rimsulfuron, primisulfuron-methyl, glyphosate and gluphosinate-ammonium, when applied at normal agricultural rates (20 mg a.i./g soil), though did not lead to any significant effect on soil microbial activity but 200 mg a.i./g soil of atrazine, terbuthylazine, rimsulfuron and primisulfuron-methyl significantly decreased soil microbial activity (Accinelli, 2002).

One of the major problems in the production of successful legume crops around the world is the emergence of weeds. Many species of legumes are slow to establish from seeds as their seedlings are relatively non competitive with weeds. These weeds, if not controlled can cause total failure of legume crop stands. And hence, herbicides are frequently used to prevent lossess from weeds in high input agricultural practices. However, many of the herbicides that are effective in controlling the common weeds can not be used in legume crops since they will also kill or severely damage legumes. The emergence of new and effective herbicides of different chemical groups in the commercial markets has however, provided farmers an opportunity to augment the productivity of crops (Table 5). Also, the application of herbicides has led to reduction in soil erosion, labour and energy requirements and cost of producing foods. Despite of the known adverse effects of certain chemicals, legumes are exposed to varying level of these

herbicides, which may be potentially hazardous and cause a major threat to the survival of both symbiotic and asymbiotic N_2 fixing organisms and various crops. Generally, herbicides action on plants involves two phases- (i) movement of herbicides to the target site and (ii) the metabolic consequences resulting from interaction at the site. The first step starts with the uptake of herbicides by the plants, either directly through foliage or via the roots. Entry of herbicides into the plants is quickly followed by a series of events that precede the arrival of herbicides at its site of action. These include entry into cells, diffusion over relatively short distance, long distance transport, metabolic conversion and entry into sub cellular organelles. The interaction of herbicides at the target site can be viewed as first step in phase 2 which is followed by a series of toxic consequences resulting in the death of plant (Fig. 11).

In this context, effects of various herbicides on the crops have concentrated solely on either plant effects or on bacterial response (Moorman, 1989). For instance, grape plants treated with herbicide (e.g., flazasulfuron) showed yellow leaves suggesting the impairment of photosynthetic activity. Following flazasulfuron application, leaf gas exchanges and photosynthetic pigment concentrations were decreased by 85 and 88% respectively. consequently the leaf plastids became disorganized. Moreover, the herbicide substantially decreased the leaf starch and soluble carbohydrate, by 74 and 90%, respectively (Magne et al., 2006). For legumes, fluchloralin is reported to enhance the nitrification rates in chickea, especially, at low rates of application (Aamil et al., 2004) while, glyphosate, is shown to reduce nitrogenase activity and nodule numbers in subterranean clover (*Trifolium repens*) (Eberbach and Douglas, 1983). Cakmak et al. (2009) reported that in greenhouse, glyphosate at the rates between 0.06 and 1.2% of the recommended dose significantly reduced chlorophyll, shoots dry weight, mineral nutrients (Ca, Mg, Fe and Mn) in both leaves and seeds of non-glyphosate resistant soybean (*Glycine max*, L.) plants and suggested that glyphosate may interfere with uptake and retranslocation of Ca, Mg, Fe and Mn, most probably by binding and thus immobilizing them. The decrease in Fe, Mn, Ca and Mg minerals of seeds due to glyphosate application may affect seed quality. Moreover, Pline et al. (2002) determined relative tissue sensitivity in glyphosate-resistant (GR) and non-GR cotton (*Gossypium* sp.) seedlings to technical grade glyphosate. Glyphosate inhibited the growth of non-GR cotton cotyledons, hypocotyls, and roots 50% at concentrations of 23, 69, and 27 μM glyphosate, respectively. In

contrast, growth of GR cotton cotyledons, hypocotyls, and roots was inhibited by 50% at 3.5, 8, and 5-fold greater glyphosate concentrations, respectively, than non-GR cotton tissues.

Table 5 Examples of herbicides and their mode of action (MoA)

MoA groups	Chemistry	Example (s)
Contact, desiccant		
Photosystem I (electron transport)	Desiccants	Diquat, paraquat
Cell membrane disruption	Dinitrophenol	DNOC
Unknown mechanisms	Organoarsenical	MSMA (methyl arsonic acid)
Multi-site soil sterilants	Varius	Dezomet, metam
Desiccants etc.	Inorganic	Sodium chlorate, sulphuric acid
Systemic		
Inhibits fatty acid synthesis (ACCase inhibitors)	'FOP' hebicides	Clodinafop-propargyl, fluazifop-P-butyl
	'DIM' herbicides	Clethodim, cycloxydim
	'DEN' herbicides	Sethoxydim, tralkoxydim
Inhibits plant amino acid synthesis: acetolactate synthase	Imidazolinones, Sulfonylureas	Pyriftalid, bensulfuron- methyl, trifloxysulfuron-sodium
Inhibits photosynthesis (photosystem II)	Triazines,Triazinones	Atrazine, metribuzin
	substituted ureas	Diuron, isoproturon
	nitriles	Bromoxynil, ioxynil
Inhibits protoporphyrinogen oxidase, leading to irreversible cell membrane damage	Diphenylethers	Oxyfluorfen, oxadiazon
Bleaching: inhibition of carotenoid biosynthesis		
-at the phytoene desaturase step (PDS)	Pyridazinones	Norflurazon, diflufenican
-of 4-hydroxyphenyl-pyruvate-dioxygenase (4-HPPD)	Triketones	Mesotrione
-inhibition of lycopene cyclase and unknown target	Triazole	Amitrole, clomazone
Inhibition of essential aromatic amino acid synthesis (EPSPS) in chlororplasts	Organophosphate glycene	Glyphosate, sulfosate
Glutamine synthetase inhibitor: accumulates ammonium ions, inhibits photosynthesis	Phosphinic acid	Glufosinate-ammonium
Inhibition of dihydropterate synthase causing slow chlorosis	Carbamate	Asulam
Inhibition of mitosis and cell division	Dintroanilines	Pendimethalin, trifluralin
	Carbamate	Chlorpropham
	Chloracetamides	Dimethenamid, butachlor
Inhibition of cell wall synthesis	Various	Dichlobenil, isoxaben
Inhibition of lipid synthesis	Thiocarbamates	Ethofumesate, thiobencarb
Synthetic auxins	Phenoxy-carboxylic-acids	2,4-D, MCPA, MCPB
Inhibits auxin transport	Phthalamate	Naptalam

Adapted from http://www.plantprotection.org/hrac

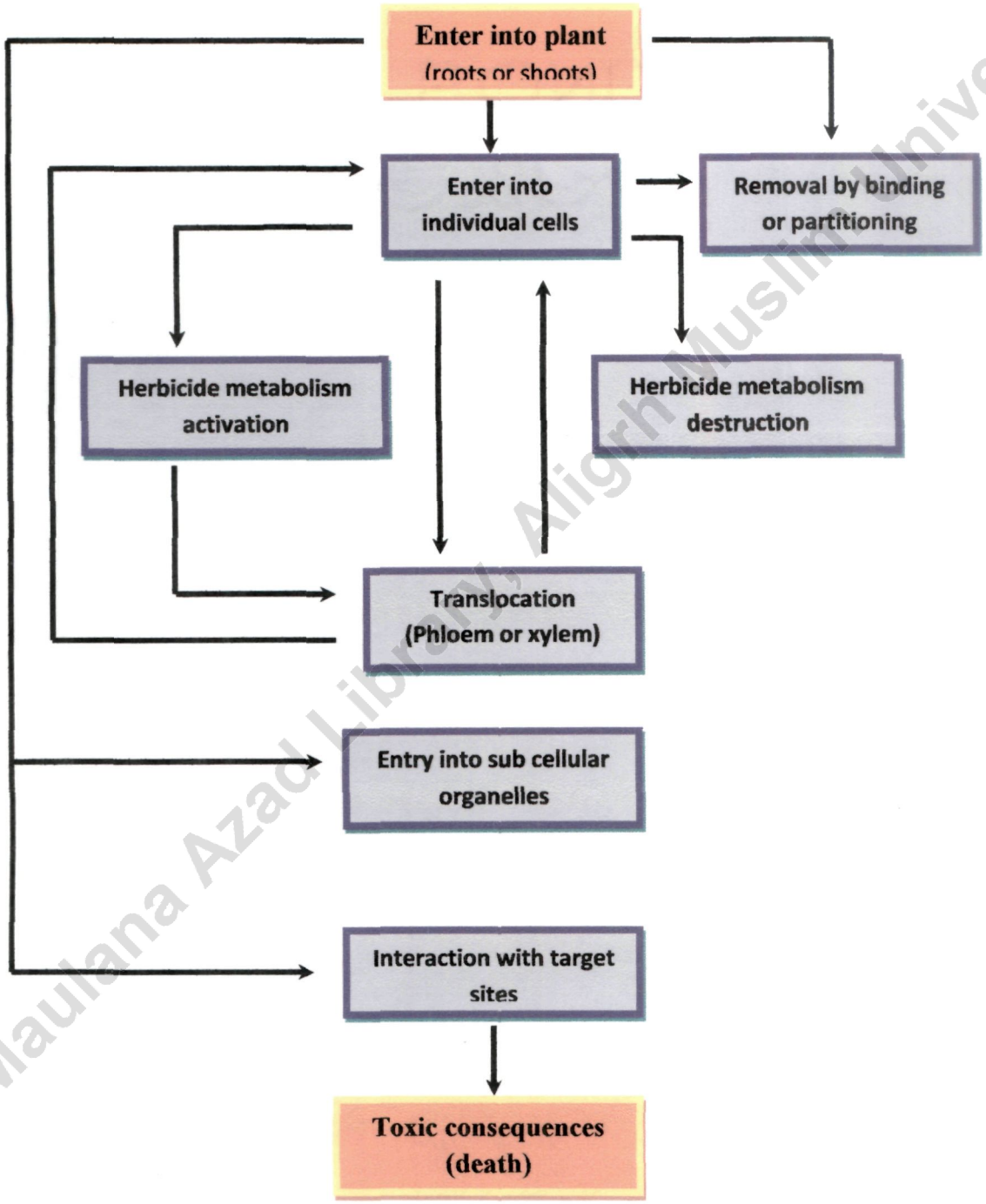


Fig. 11: Flow chart showing the sequence of events from herbicide entry into a plant to the death of the plant

Similarly, Tesfamariam et al. (2009) reported that sunflower (*Helianthus annuus* L.) seedling growth and biomass production was strongly impaired by glyphosate pre-sowing treatments. Generally, the detrimental effects were more pronounced after glyphosate weed application [plant residues of glyphosate-treated weeds (model plant perennial rye grass, *Lolium perenne* L.)] (90% biomass reduction) compared with direct soil application (55–70% biomass reduction). The inhibitory effects on seedling growth were associated with an impairment of the manganese-nutritional status and a corresponding increase in shikimate accumulation in the root tissue as physiological indicator for glyphosate toxicity. Daramola and Adebayo (1981) evaluated the effects of the application of three herbicide formulations namely: preforan (2,4-dinitro-4-trifluoromethyl-diphenyl ether), dacthal (dimethyl 2,3,5,6-tetrachloroterepthalate) and dual (2,ethyl-6-methyl-N-(2-methoxy-1-ethyl-a-chloro-acetanilide) on legume-*Rhizobium* symbiosis by incorporating these herbicide formulations into plate agar cultures of two *Rhizobium* strains and into soil carrying cowpea (*Vigna unguiculata* (L.) Walp.). The Results of this study indicated that dual is bactericidal giving the lowest mean cell count 3.04×10^6 for strain TAL 385 in agar culture, compared to 3.51 and 3.43×10^6 obtained for dacthal and preforan respectively. In soils system, while the highest rate of dacthal and preforan merely decreased nodulation, the highest rate of dual completely killed the plants within 14 days after planting. The rates of herbicide application and the herbicide type were both important factors which affected vital parameters of the legume-*Rhizobium* symbiosis in soil culture.

In other study, glyphosate applied at recommended field rates had no effect on *Bradyrhizobium japonicum* colonization upon soybean roots, or on soybean foliar tissue. N_2-fixation was greater for glyphosate-treated soybean plants than for untreated-plants but only when glyphosate was applied at the first trifoliate soybean growth stage (Powell et al., 2009). These results deviate from previous studies estimating the effect of glyphosate on the rhizobial symbiosis, some of which observed negative effects on rhizobial colonization and/or N_2-fixation. In another study, Arregui et al. (2006) concluded that soil-applied herbicides may be beneficial for glyphosate-tolerant crops reducing early season competition of weeds, particularly those inherently more tolerant to glyphosate. Moreover, use of glyphosate in conservation tillage and other tillage systems on sandy loam soils had no detrimental effect on soil biological properties (Carter et al., 2007).

Table 6: Impact of selected herbicides on soil biota

Herbicides	Effects	References
Glyphosate, hexazinone	Short-term effects of glyphosate on both fungal and bacterial counts. Growth of ectomycorrhizal fungi reduced.	Chakravarty and Chatarpaul (2006)
Simazine	Compared with control soil, simazine (at 10, 50, 100, 200, and 300 µg/g soil) did not affect bacterial populations, fungi, aerobic dinitrogen-fixing bacteria, denitrifying bacteria, and nitrogenase activity. Nitrifying bacteria were decreased at concentrations of 50 to 300 µg/g. The negative effects observed on nitrifying bacteria in soil amended with simazine were particularly evident after a second application of herbicide, showing that these microorganisms cannot tolerate certain concentrations of simazine.	Martinez-Toledo et al. (2005)
Agroxone, Atranex 50SC, 2,4-Damine	2, 4-Damine was the most toxic of the three herbicides and *Azotobacter vinelandii* was found to be most sensitive to the herbicides. The percentage survival decreased with increased concentration of herbicides and days for *Rhizobium phaseoli* and *Azotobacter vinelandii* while an initial reduction in population was followed by increased percentage survival of organisms for *Bacillus subtilis*.	Adeleye et al. (2004)
Glyphosate	Reduced bacterial populations. Fungal and actinomycetal population increased. Increased rate of glyphosate degradation over time.	Araujo et al. (2003)
Glyphosate	Short-term changes to community structure. Increased microbial activity and no long-term changes to community structure	Busse et al. (2001)
Glyphosate and paraquat	Activated urease and invertase soil enzymes, but suppressed phosphatase enzyme	Sannino and Gianfreda (2001)
Pendimethalin	Soil nematodes and other invertebrates reduced, Plant-*Rhizobium* symbiosis affected	Strandberg and Scott-Fordsmand (2004)
Atrazine and metachlor	Altered community structure of several groups of bacteria and actinomycetes	Seghers et al. (2003)
Atrazine	Significantly activated soil urease activity, and suppressed invertase enzyme.	Sannino and Gianfreda (2001)
Butachlor	Significant effects on earthworms and soil health	Panda and Sahu (2004)
Oxyfluorfen	Stimulated microbial populations, and increased availability of phosphorus in rhizosphere soil.	Das et al. (2003b)

Similarly, the growth of N_2 fixing pea-rhizobium (*Rhizobium leguminosarum*) was also influenced when treated with herbicides terbutryn/terbuthylazine, trietazino/simazine, prometryn and bentazone. Terbutryn/terbuthylazino, trietazne/simazine and prometryn had an adverse effect on the growth of rhizobia at concentrations not normally expected to occur under field conditions. Bentazone was however, found safe to rhizobia at the recommended rates. Furthermore, it was suggested that adverse effects of these herbicides on nodulation and nitrogen fixation of pea were not due to their effects on rhizobia alone but due to their deleterious effects on the plant growth itself (Singh and Wright, 2002a).

In another study, treatment with chlorotoluron at concentrations of 0–25 mg/kg soil induced the accumulation of reactive oxygen species (singlet oxygen) and H_2O_2 in leaves leading to the peroxidation of plasma membrane lipids in wheat (*Triticum aestivum*). In addition, the endogenous proline level was increased significantly in roots and leaves and activities of the antioxidant enzymes, such as superoxide dismutase, peroxidase, and ascorbate peroxidase were increased while the activity of catalase was generally suppressed under the chlorotoluron exposure (Song et al., 2007). In other report, Khan et al. (2006a), observed a deleterious effect of the pre-emergenct application of atrazine, isoproturon, metribuzin and sulfosulfuron on plant vigour, nodulation, chlorophyll content, seed yield and grain protein of greengram, inoculated with *Bradyrhizobium* sp. (vigna). Among the herbicides, atrazine and metribuzin showed a larger phytotoxic effect on the measured parameters. In comparison, methabenzthiazuron, terbutryn, and linuron at lower dose rate had no significant adverse effect on plant vigor, number of nodules formed, nitrogenase activity, chlorophyll contents, nitrogen uptake, and seed yield of chickpea inoculated with species of *Mesorhizobium*, grown in sandy clay loam soil, in a controlled environment. These herbicides at rates greater than recommended dose, however, significantly reduced the assayed parameters. The toxicity of all dose rates of herbicides on grain yield increased in the following order: control = linuron > methabenzthiazuron > terbutryn. Furthermore, nodulation (nodule number per plant and their dry mass) and chlorophyll contents per plant decreased consistently with increasing concentration of each herbicide, except linuron, which improved nodulation of chickpea (Khan et al., 2006b). Under greenhouse conditions, the effects of soil applications of bentazone, isoproturon, fluchloralin and 2,4-D on plant vitality, chlorophyll content, N and protein content, nodulation and seed production in chickpea, inoculated with *Mesorhizobium ciceri* were variable. Applying ten times the recommended rates, adversely affected the plant vigor, total chlorophyll content and nitrogen content in shoots and seed production. Fluchloralin and 2,4-D gave the highest adverse effects on seed production while protein content in seeds increased significantly following herbicide applications but decreased with an increase in application rates. The ten times the recommended rates of bentazone and 2,4-D however, completely decreased nodulation on the root systems of chickpea plants (Khan et al., 2004). It is also reported that the soybean nitrogen fixing symbiont, possesses a glyphosate-sensitive enzyme and upon exposure to glyphosate, accumulates shikimic acid and

hydroxybenzoic acids, such as protocatechuic acid (PCA), accompanied with growth inhibition and death at high concentrations. In a series of greenhouse and field experiments, glyphosate inhibited nodulation and nodule leghemoglobin content of glyphosate-resistant (GR) soybean. Eventhough, glyphosate accumulated in nodules of field-grown GR soybean, yet its effect on nitrogenase activity of GR soybean was inconsistent in field trials. In greenhouse studies, nitrogenase activity of GR soybean following glyphosate application was transiently inhibited especially in early growth stages, with the greatest inhibition occurring under moisture stress. Studies using bacteroid preparations showed that the level of glyphosate inhibition of bacteroid nitrogenase activity was related to *in vitro* glyphosate sensitivity of the strains (Zablotowicz and Reddy, 2004).

2.3.2. Insecticidal impact on rhizobacteria and crops

In modern agriculture practices, application of insecticides belonging to diverse chemical groups (Table 7) as seed and soil treatments has become a common practice to combat insect pests. When applied, such insecticides accumulate in soils and affect directly or indirectly the soil enzyme activities and physiological activities of non-target soil microbiota (Table 8) leading thereby to the losses in fertility of soils. In this section, an attempt is made to highlight the adverse effects of insecticides on soil microorganisms and certain crops.

In a study, application of monocrotophos, quinalphos, and cypermethrin at different rates used either singly or in combination to a black clay soil, significantly enhanced the proliferation of bacteria and fungi and the soil dehydrogenase activity even at the highest level of 25 $\mu g\ g^{-1}$ (Gundi et al., 2005). The mixture of monocrotophos or quinalphos and cypermethrin showed additive, synergistic, and antagonistic effects towards bacteria and fungi and dehydrogenase activity in soil. Antagonistic interactions were, however, more pronounced for soil microflora and dehydrogenase activity when the two (monocrotophos or quinalphos + cypermethrin) insecticides were added together to the soil at highest level (25 + 25 $\mu g/g$). Synergistic or additive responses, on the other hand, occurred at lower level with the same combination of insecticides in soil. However, some insecticide tolerant strains of PGPR are also known. For example, Nazarian and Mousawi (2005) identified strains belonging to *Pseudomonas* and *Flavobacterium* species whose tolerant concentrations of different organophosphorus pesticides

were 2.5, 4 and 8 g/L of guthion, methyl parathion and dimethoate, respectively. The resistance in these bacteria against such pesticides was probably due to the presence of organophosphorous degrading plasmids that bears the ability to express hydrolytic enzymes.

Table 7: Examples of insecticides and their mode of action

MoA groups	Chemistry	Examples
Acetylcholinesterase inhibitors	Carbamates	Aldicarb, carbaryl, carbofuran, propoxur, carbosulfan
	Organophosphates	Phorate, chlorpyrifos, omethoate, parathion, methmidophos, malathion, diazinon
GABA-gated chloride channel antagonists	Cyclodienes and other organochlorines (OC)	Lindane, aldrin, endosulfan
	Phenylpyrazoles (fiproles)	Fipronil
Sodium channel modulators	OC	DDT
Acetylcholine receptor agonists	Neonicotinoids	Imidacloprid, thiamethoxam
Acetylcholine receptor agonists allosteric	Spinosyns	Spinosad
Voltage dependent sodium channel blocker	Oxadiazine	Indoxacarb

Adapted from http://www.irac-online.org/

Furthermore, Vasileva and Ilieva (2007) carried out pot trials to determine the effect of pre-sowing treatment of seeds with insecticides promet 400 SK (furathiocarb) at the dose of 3 L, and carbodan 35 ST (carbofuran) at the dose of 1, 2 and 3 L/100 kg seeds on the nodulating ability, nitrate reductase activity and plastid pigments content of lucerne (cv. obnova). It was found that the tested insecticides did not depress the nodulation, instead the nodule numbers and specific nodulation ability of carbodan 35 ST (3 L/100 kg seeds) treated plants inceased by 23% and 7%, respectively, compared to control. The root length for the variants with presowing treatment of seeds was higher as compared to the control by 7 to 26%. The variant with carbodan at the doses of 2 and 3 L/100 kg seeds and Promet increased nitrate reductase activity in roots, and that with carbodan at the dose of 1 L/100 kg seeds- in the leaves. Total content of plastid pigments increased in all variants with carbodan and was lower than the untreated control in the variant with Promet.

In contrast, Das et al., (2003a) investigated effects of phorate and carbofuran at rates of 1.5 and 1 kg a.i./ha respectively on the population and distribution of bacteria, actinomycetes and fungi as well as the persistence of the insecticidal residues in rhizosphere soils of rice (*Oryza sativa* L., variety IR-50). Application of the insecticides stimulated the population of bacteria,

actinomycetes and fungi in the rhizosphere soils: the stimulation was more pronounced with phorate as compared to carbofuran. Both the insecticides however, did not markedly affect the numbers of *Streptomyces* and *Nocardia* in the rhizosphere soils. The growth of *Bacillus*, *Escherichia*, *Flavobacterium*, *Micromionospora*, *Penicillium*, *Aspergillus* and *Trichoderma* with phorate and that of *Bacillus*, *Corynebacterium*, *Flavobacterium*, *Aspergillus* and *Phytophthora* with carbofuran were increased. On the other hand, the numbers of *Staphylococcus*, *Micrococcus*, *Fusarium*, *Humicola* and *Rhizopus* under phorate stress and *Pseudomonas*, *Staphylococcus*, *Micrococcus*, *Klebsiella*, *Fusarium*, *Humicola* and *Rhizopus* under carbofuran stress were inhibited. Similarly, Wani et al. (2005) reported that phorate at a rate of 100 and 500 µg /ml substantially reduced the IAA production by phosphate solubilizing bacteria belonging to genera *Serratia*, *Pseudomonas* and *Bacillus* isolated from various rhizospheric soils while P solubilizing activity of PSB was marginally affected.

The effect of lindane on microbial populations was analyzed by Rodrı´guez and Toranzos (2003) in soil with a history of contamination with various chemicals, including pesticides. Soil microcosms were amended with 100 mg lindane/kg soil and microbial populations were monitored for 70 days. Results showed a reduction of 50% in bacterial cell concentration in lindane-amended microcosms during the second week of the experiment. Overall, no effect of lindane was observed on the metabolic versatility and genetic diversity in these soils, demonstrating the ability of the bacterial populations to tolerate the pressure generated by the addition of pesticides. In another report, pencycuron at field rate (FR), 2FR and 10FR affected the microbial biomass C (MBC), soil ergosterol content and fluorescein diacetate hydrolyzing activity (FDHA) differentially. Change in microbial metabolic quotient (qCO_2) and microbial respiration quotient, indicated pencycuron induced disturbance at 10FR. This study revealed that the metabolically activated microbial population was more suppressed compared to the dormant population (Pal et al., 2006). Das et al., (2005) observed that the application of phorate and carbofuran at their recommended field rates in general, induced growth and development of bacteria, actinomycetes, fungi, N_2-fixing bacteria and phosphate solubilizing microorganisms in both laterite and alluvial soils.

Table 8: Impacts of selected insecticides on soil biota

Insecticides	Effects	References
Malathion, dimethoate, phorate	Aerobic bacteria, among all groups of microflora, were most adversely affected by all insecticides at normal or four times more of normal rate and phorate was found to be most toxic	Aamil et al. (2005)
Chlorpyrifos	Reduced bacterial numbers, but significantly increased fungal numbers	Pandey and Singh (2004)
Carbofuran	Significant impacts on acetylcholinesterase activity in earthworms.	Panda and Sahu (2004)
Dimethoate	Short-term reduction in microarthropod numbers (Collembola), but recovery in numbers after time.	Martikainen et al. (1998)
DDT	Reduced bacterial and soil algal populations, but may have increased fungal counts	Megharaj et al. (2000)
Malathion	Short-term impacts on earthworm population	Panda and Sahu (1999)
BHC, phorate, carbofuran, and fenvalerate	stimulated the proliferation of aerobic nonsymbiotic N_2-fixing bacteria and phosphate-solubilizing microorganisms and also their biochemical activities, such as nonsymbiotic N_2-fixing and phosphate-solubilizing capacities, which resulted in greater release of available N (NH_4^+ and NO_3^-) and P in soil	Das and Mukherjee (2000)

The stimulation was more pronounced with phorate as compared to carbofuran. Application of phorate stimulated substantially the population of fungi in laterite and actinomycetes in alluvial soil. Carbofuran on the other hand, augmented fungi and N_2-fixing bacteria in laterite and actinomycetes in alluvial soil. Bacterial population was inhibited due to the application of carbofuran in alluvial soil. In contrast, the effect of increasing rates of lindane (156.0, 244.0 and 312.0 g/ha), unden (propoxur) (125.0, 187.5 and 250.0 g/ha), dithane and karate (166.6, 209.8 and 333.3 g/ha) on garden eggs (*Solanum melongena*), okro (*Abelmoschus esculentus*) and tomatoes (*Lycopersicum esculentus*) was studied by Glover-Amengor and Tetteh (2008). Yields of garden eggs were suppressed by all the rates of lindane applied. In tomatoes, lower rates of lindane increased yields whereas the higher rates suppressed yields lower than the control. In okro yields were higher than the control at all levels of lindane applied though yield increments were low. Unden application had the highest effect on garden egg yields followed by tomatoes and least on okro. In the garden egg and tomato treatments, increasing concentration of unden resulted in decreasing yields though yields were higher in the control plots. The optimum unden rate for garden egg and tomato was U20 (i.e. 125.0 g/ha). Increasing rates of unden on okro did not have any significant effect. Pesticide application reduced the fungal population by 50-70% while bacterial population was declined by 23–38% in soils. In general, dithane suppressed the

bacterial population considerably whereas karate suppressed the fungal populations. Lindane did not have any advantage over other pesticides as it caused the least increase in yield.

Singh and Singh (2006) evaluated the impacts of diazinon, imidacloprid and lindane treatments on ammonium, nitrate, and nitrite nitrogen and nitrate reductase enzyme activities in groundnut field for three consecutive years (1997 to 1999). Diazinon was applied for both seed- and soil-treatments but imidacloprid and lindane were used for seed treatments only at recommended rates. Diazinon residues persisted for 60 days in both the cases. Average half-lives ($t_{1/2}$) of diazinon were found 29.3 and 34.8 days in seed and soil treatments, respectively. In diazinon seed treatment, NH_4^+, NO_3^-, and NO_2^- nitrogen and nitrate reductase activity were not affected. Whereas, diazinon soil treatment indicated significant increase in NH_4^+-N in a one day sample, which continued until 90 days. Some declines in NO_3^- N were found from 15 to 60 days. Along with this decline, significant increases in NO^-_2 N and nitrate reductase activity were found between 1 and 30 days. Imidacloprid and lindane persisted for 90 and 120 days with average half-lives ($t_{1/2}$) of 40.9 and 53.3 days, respectively. Within 90 days, imidacloprid residues were lost by 73.17% to 82.49% while such losses for lindane residues were found as 78.19% to 79.86 % within 120 days. In imidacloprid seed-treated field, stimulation of NO_3^- N and the decline in NH_4^+, NO_2^--N and nitrate reductase enzyme activity were observed between 15 to 90 days. However, lindane seed treatment indicated significant increases in NH_4^+-N, NO^-_2 -N and nitrate reductase activity and some adverse effects on NO^-_3 N between 15 and 90 days. Fox et al., (2007) concluded through study on interaction of agrochemicals with crop plants that organochlorine pesticides, agrichemicals, and environmental contaminants induces a symbiotic phenotype of inhibited or delayed recruitment of rhizobia bacteria to host plant roots, fewer root nodules produced, lower rates of nitrogenase activity, and a reduction in overall plant yield at the time of harvest. Moreover, Evans et al. (1991) reorted that omethoate was toxic to some *Rhizobium* strains on direct contact when diffused through agar seeded with these bacteria or mixed in broth cultures containing the bacteria. Omethoate mixed with peat-based legume inoculant and applied to seed of subterranean clover or lucerne significantly reduced the number of nodules formed over three weeks on seedlings grown in pots of sand, compared with inoculated controls. Rhizobia numbers were reduced markedly by mixing with omethoate. Seed pretreatment with omethoate before inoculation had no effect on nodule number (9-11 weeks

after sowing), compared with inoculated controls. In other experiment, Evans et al. (1993) found that the effectiveness of inoculation with *Rhizobium meliloti* was significantly reduced when inoculant was applied to seed pre-treated with omethoate. Generally the nodule number and shoot mass per plant were reduced by 6 and 22%, compared to plants having no omethoate treatment.

2.3.3. Fungicidal toxicity to plant growth promoting rhizobacteria and agronomic crops

Fungicides of different chemical families (Table 9) commonly used as seed treatments to prevent seed-borne diseases ultimately reach the soil and influence the activities of soil microbes (Cernohlavkova et al., 2009) and plant productivities (Hashem et al., 1997). Further, some of the fungicides that give very good protection to one crop may have phytotoxic effect on non-target microorganisms, such as, *Rhizobium* spp. (Ruiz-Sainz et al., 1984) and the succeeding crop. However, very few attempts have been made to understand the effect of these chemicals on legume-*Rhizobium* symbiosis. In the following sections, an attempt is made to highlight the impact of fungicides on legume-*Rhizobium* symbiosis.

Effect of some selective fungicides on soil microflora, their enzymatic and other activities is summarized in Table 10. Information on the compatibility of *Rhizobium* spp. with seed protectant chemicals is controversial because of variations in the methods used and lack of quantitative data. In a study, Castro et al. (1997) determined the influence of the fungicide mancozeb (ethylenebis-dithiocarbamate), at recommended doses, on the growth, survival and symbiotic properties of *Rhizobium* sp. infecting peanut plants under laboratory and field conditions. The results indicated that mancozeb decreased growth in pure culture by 50% of both *Rhizobium* sp. USDA 3187 and a strain isolated from peanut nodules. However, no differences were found in peanut seed yields under field conditions. These results suggest that the soil environment could reduce the probability of the direct, harmful effects of mancozeb on bacterial growth. Recently, Kaur et al., (2007) while investigating the effect of fungicides (e.g. captan and carbendazim) on the growth of *Rhizobium japonicum* found that carbendazim is more toxic to the nodule bacterium than captan. Some fungicide seed treatments are also reported to hinder nodule development, and thereby reduce legume yields. For example, The effects of carbendazim, captan, thiram and mancozeb on plant vitality, chlorophyll, N uptake, protein content, nodulation

and seed yield in chickpea were assessed by Aamil et al., (2004) in a controlled environment. Seeds treated with fungicides at one and 1.5g a.i./kg seeds had no significant adverse effect on plant vigor , seed yield, N and protein content. In contrast, captan, thiram and mancozeb applied at 2g a.i./kg seeds significantly reduced the measured parameters. In general, the toxicity of fungicides in terms of seed yield increased in the following order: control = carbendazim > thiram > captan > mancozeb. Total chlorophyll content in foliage declined consistently with increasing dose rates of fungicides and application days. Seeds treated with lower rates of fungicides significantly increased nodulation (nodule number sand their dry mass/plant) and were compatible with chickpea inoculum. Although, carbendazim at g a.i./kg seeds had no phytotoxic effect assessed under greenhouse conditions., it significantly reduced the chlorophyll content, nodulation (60 days) and N content in shoots. Similarly, Rennie et al., (1985) treated pea, lentil and faba bean (*Vicia faba*) with the fungicide captan [*cis-N*-(trichloromethyl) thio-4-cyclohexane-1,2-dicarboximide], and inoculated seed with a peat-based *Rhizobium leguminosarum* inoculant. Under the southern Alberta growing conditions, captan was found to reduce root nodule number and nitrogenase activity, as well as shoot yield at anthesis. Furthermore, fungicides may have a negative impact on the synergistic relationship between rhizobia and root-associated mycorrhizal fungi (Redente and Reeves, 1981). For example, Dunfield et al., (2000) assessed the effects of the captan and thiram at rates of 0.25–2 g a.i. kg^{-1} seed on the survival and phenotypic characteristics of *Rhizobium leguminosarum* bv. *viceae*, strain C1. Captan and thiram significantly reduced the numbers of rhizobia recovered from seed and altered the FAME and Biolog profiles of recovered rhizobia. However, only the highest concentrations of captan affected nodulation and legume growth. Contact with some seed-applied fungicides can significantly alter phenotypic characteristics of rhizobia.

The survival of *Rhizobium ciceri* on chickpea seed, treated separately with commercial fungicides, (i.e., apron, arrest 75W, crown and captan) was examined by Kyei-Boahen et al. (2001) under laboratory conditions using standard serial dilution and plate count techniques. The resulting effects of fungicide–*Rhizobium* interactions on nodulation, N_2 fixation, and plant growth were assessed in a controlled environment. Fungicide treatment decreased the number of viable rhizobia on the seed. In general, the toxicity of the fungicides in terms of rhizobial viability increased in the following order: control = crown < arrest = apron < captan. Although,

crown had no effect on rhizobial viability, it significantly reduced nodulation, percent N derived from the atmosphere (%Ndfa), and shoot dry matter. Seed treated with arrest and captan decreased nodule dry weight and %Ndfa, but only arrest reduced dry matter yield. Apron had no effect on any of the parameters measured at the early pod-filling stage and was compatible with the chickpea inoculum. In a study, commercial fungicides like, quinolate Pro (carbendazim and oxine copper), Vitavax 200FF (carboxin and thiram), and Monceren (pencycuron) had a small effect on the survival of *Bradyrhizobium japonicum* and on the nodulation and yield of soybeans. While, Germipro UFB (carbendazim and iprodione), Apron 35J (metalaxyl), and Tachigaren (hymexazol) decreased *B. japonicum* survival and the nodulation and yield of soybeans (Revellin, 1993).

Table 9: Examples of fungicides and their mode of action

MoA groups	Chemistry	Examples
Multiple site of action (M)		
M1	Copper fungicides	Copper ammonium carbonate, Copper hydroxide
M2	Sulphur	Sulfur
M3	Dithiocarbamates	Mancozeb, thiram, ferbam
M4	Pthalimides	Captan, folpet
M5	Chloronitriles	Chlorothalonil
M5	Sulphamides	Dichlofluanid, tolylfluanid
M6	Guanidines	dodine
Systemic		
Disruption of fungal RNA polymerase Fungal DNA inhibition	Phenylamides	Benalaxyl, metalaxyl Hymexazole
Inhibition of beta tubulin assembly	Benzamidazoles	Benomyl, carbendazim
Inhibition of cell division		Pencycuron
Uncouplers of oxidative phosphorylation		Dinocap
ATP synthase inhibitors	Organo-tin compounds	Fentin acetate
Protein synthesis inhibitors	Anilinopyrimidines	cyprodinil
Membrane phospholipid synthesis inhibitors	Phosphrothiolates,	Edifenophos
Lipid peroxidation	Aromatic hydrocarbon	Tolclofos-methyl
De-methylation inhibitors	Triazoles	Hexaconazole, tebuconazole

Adapted from http://www.frac.info/

Lennox and Alexander (1981) observed that the number and weight of pods and the weight and nitrogen content of the tops of beans (*Phaseolus vulgaris*) derived from seeds inoculated with a thiram resistant strain of *Rhizobium phaseoli* were increased if the seeds were treated with thiram before sowing in soil. A greater percentage of the nodules on 21-day old plants were derived from the resistant strain, more nodules were formed, and these nodules were more effective in the presence of the fungicide than in its absence. These differences in nodule

numbers were no longer present in 56-day old plants, and only a small percentage of the nodules contained the resistant strain. The abundance of the fungicide-tolerant *R. phaseoli* increased rapidly soon after planting the seed and subsequently fell markedly, but the rate of decline was less if the seeds were treated with the chemical. Also, Gaind et al. (2007) tested survival of *Mesorhizobium ciceri* (SP4) and *Azotobacter chroococcum* (CBD-15 and M4) on chickpea seeds treated with fungicides (bavistin and thiram). Furthermore, the effect of such fungicides was evaluated on the survival of phosphate solubilizing bacteria (PSB), *Pseudomonas striata* and *Bacillus polymyxa* on two cultivars (Arkel and BV) of pea seeds treated with thiram. The viable populations of all the tested strains of diazotrophs and PSB declined on prolonged contact with fungicides. However, PSB differed in their viable population even with the cultivar. Under field conditions, thiram adversely affected the performance of *Mesorhizobium ciceri* (SP4) and *A. chroococcum* (M4) strains, leading to a reduced vegetative growth (root and shoot biomass) and grain yields, compared to bavistin treated and culture inoculated treatment. *Azotobacter chroococcum* (CBD-15) however, performed better in the presence of thiram compared to bavistin. Guene et al., (2003) conducted a field experiment using ^{15}N isotope dilution technique and the non nodulating soybean variety m129 as a reference plant to test the compatibility of Dichlorofenthion-thiram (DCT) fungicide to the inoculation of common bean Paulista variety with both *Rhizobium etli* ISRA 353 and *R. tropici* strain ISRA 554. They observed that nodulation was not induced by *R. etli* ISRA 353 and hence, nitrogen fixation did not occur. With *R. tropici* ISRA 554, a decrease in nodulation was observed, but nitrogen fixation was not significantly different compared to that of the non DCT-treated common bean. In a follow up study, Bikrol et al. (2005) investigated the effect of various concentrations of thiram on *Glycine max-Rhizobium* interaction. Thiram concentration beyond 500 µg/ml was found as highly toxic with respect to plant growth factors and rhizobial infection to the soybean. The nodulation, nodule dry mass and nitrogenase activity were recorded maximum at 100 µg/ml of thiram. This study thus may help in determining the threshold level of fungicide for soybean seed dressing for effective nitrogen fixation and crop yield. Untiedt and Blanke (2004) assessed the impact of mixture of fungicides (mancozeb, flusilazol and dithianon) and insecticide (oxydemeton-methyl or pirimicarb) at doses commonly used in agronomic practices on photosynthesis and dark respiration in two seasons with respect to the potential stress they impose on apple tree. The

mixture of fungicides mancozeb and flusilazol when used with the insecticide oxydemeton-methyl, reduced whole tree canopy CO_2 assimilation. This reduction in whole canopy photosynthesis declined with time, restoring most of the original photosynthetic potential within 3–5% in 3 days, indicating acceptable phytotoxicity. This fungicide/insecticide mixture increased the dark respiration up to 72% in the night after application, thereby drastically affecting the tree's carbon balance in an adverse way. In contrast, the fungicide dithianon when used with the insecticide pirimicarb, decreased dark respiration by 15–21% with reductions in canopy photosynthesis in the order of 6–9%.

Table 10: Effects of selected fungicides on soil biota

Fungicides	Effects	References
Phenyl mercuric acetate, pentachloro-nitrobenzene, benomyl, captan	Phenyl mercuric acetate completely inhibited the soil bacteria and fungi at all rates of application, pentachloro-nitrobenzene decrased severely the population size of actinomycetes and protozoa, Benomyl and captan showed comparatively lower toxicity to soil microflora	Ojo et al. (2007)
Copper	Earthworm populations avoid soils with concentrations as low as 34mg/kg. Lack of breakdown of organic carbon. Potential long-term implications reported	Van Zwieten et al. (2004)
Copper	Increased respiration indicating microbial stress. Significantly reduced microbial biomass	Merrington et al. (2002)
Copper	Reduced performance of soil functions resulting in reduction of DDT degradation	Gaw et al. (2003)
Metalaxyl	Reduced enzyme activity, in particular dehydrogenase, toxic to nitrogen fixers	Monkiedje et al. (2002)
Benomyl	Suppression of respiration, stimulation of dehydrogenase, effects were less noticeable with organic matter addition	Chen et al. (2001)
Benomyl	Significant long-term impacts on mycorrhizal colonization (80% reduction), reduction in fungal to bacterial ratios and nematode numbers	Smith et al. (2000)
Captan	Suppression of respiration and dehydrogenase, but increases in ammonium nitrogen	Chen et al. (2001)
Chlorothalonil	Suppression of respiration, stimulation of dehydrogenase	Chen et al. (2001)
Mancozeb	Causes a 50% decrease in *Bradyrhizobium* sp. USDA 3187 growth rate, produced biochemical alterations in membrane composition, polysaccharides and polyamines	Fabra et al. (1998)

In addition, survival and viability of *Bradyrhizobium* inoculant on fungicide-treated peanut (var. florunner) seeds and the resulting effects on nitrogen fixation, plant growth and seed yield were determined by Hashem et al., (1997) who concluded that fungicides vitavax and benomyl inhibited viability and survival of *Bradyrhizobium* on peanut seeds and in turn decreased *Bradyrhizobium*-peanut symbiosis, nitrogen fixation, plant growth and seed yield. In contrast, Muthomi et al. (2007) reported that seed treatment with fungicide, copper oxychloride,

in combination with *Rhizobium* inoculation increased seedling emergence, reduced seedling mortality and enhanced nodulation in common bean, greengram and lablab (*Lablab purpureus*).

Materials and Methods

3.1 Soil samples and microbial diversity

The samples were collected from the rhizospheric soils of chickpeas (*Cicer arietinum* L.), lentil (*Lens esculentus*), greengram (*Vigna radiata* L. Wiclzek), pea (*Pisum sativum*) and mustard (*Brassica compestris*) grown at the experimental fields of Faculty of Agricultural Sciences, Aligarh Muslim University, Aligarh, Uttar Pradesh, India,. From each site, three soil samples were collected in sterilized polythene bags (15×12 $cm.^2$). The samples were mixed well and were used to determine microbial diversity including total bacterial populations, fungal populations and phosphate solubilizing microorganisms (PSM) using standard microbiological methods (Holt et al., 1994). The soil samples were serially diluted in sterile normal saline solutions and 10 µl of diluted suspension was spread plated on nutrient agar (Appendix 1), Martin's medium (Appendix 2) and Pikovskaya (Pikovskaya, 1948) medium (Appendix 3) for total bacterial count, fungal populations and phosphate solubilizers, respectively. Each sample was replicated three times and incubated at 28 ± 2 ^{0}C for three, five and seven days for total bacteria, fungi and phosphate solubilizing microorganisms, respectively.

3.2 Isolation of nitrogen fixing bacteria

The nitrogen fixing bacteria were isolated from the nodules of chickpea, greengram, pea and lentil grown at the experimental fields of Faculty of Agricultural Sciences, Aligarh Muslim University Aligarh, U.P., India, using standard method (Somasegaran and Hoben, 1994). The nodules removed from the root system of each legume plant were surface sterilized with 2.5% sodium hypochlorite for 2 min, following a rinse in 95% ethanol (v/v) and washing six times with sterile water and squashed in normal saline solution. Nodule suspensions were diluted in normal saline solution and 10 µl of each suspension was spread plated on solid yeast extract mannitol (YEM) medium (Appendix 4) supplemented with 2.5 % Congo red indicator. The plates were incubated at 28 ± 2 ^{0}C for three to five days. The single colony was picked and streaked four times on the same medium to affirm the purity of the cultures. Isolated colonies were maintained on the YEM agar medium at 4 ^{0}C until use.

3.3 Isolation and screening of phosphate solubilizing microorganisms

The phosphate solubilizing microorganisms were isolated from the rhizospheric soils of mustard, grown at the experimental fields of Faculty of Agricultural Sciences, A. M. U., Aligarh, using Pikovskaya agar (Appendix 3) medium (Pikovskaya, 1948) by spread plate method. A 10 µl of serially diluted suspension was spread plated on solid Pikovskaya medium. Plates were

incubated at 28 ± 2 ^{0}C for seven days. The isolates showing clear halo formed within seven days around bacterial colonies were considered as phosphate solubilizers. The phosphate solubilizers were maintained on solid Pikovskaya agar medium until use.

3.4 Identification of the most promising plant growth promoting rhizobacterial strains

The plant growth promoting rhizobacteria including phosphate solubilizers and nitrogen fixers were identified using morphological and biochemical tests.

3.4.1 Morphological and biochemical properties

The isolated bacterial cultures were Gram stained (Appendix 5) and bacteria showing purple color were considered as Gram positive while those producing pink color were grouped as Gram negative. For indole test, each test isolate grown in autoclaved nutrient broth (Appendix 1), was incubated at 28±2 ^{0}C for 24-48 h. After incubation, 2-3 drops of Kovac's reagent (Appendix 6) was added to broth and the formation of red ring was considered as indole positive reaction. Autoclaved MR-VP broth (Appendix 7) inoculated with each isolate was incubated at 28±2 ^{0}C for 24-48 h. Methyl red (Appendix 8) solution was added as indicator. The development of red colour was considered as methyl red positive. Furthermore, autoclaved MR-VP broth was inoculated with test organism and incubated at 28±2 ^{0}C for 24-48 h. After incubation, Barrit's reagent (Appendix 9) was added and the development of red color indicated a positive test for Voges-Proskaur. Autoclaved Simmon's citrate agar (Appendix 10) plates were spot inoculated with test isolates and incubated at 28±2 ^{0}C for 24-48 h. Change in colour from green to blue indicated citrate utilization. Test isolates were inoculated in nutrient broth and incubated at 28 ±2 ^{0}C for 24-48 h. A 3%, H_2O_2 was added and observed for bubble formation.

Autoclaved trypticase nitrate broth (Appendix 11) tubes inoculated with test isolates were incubated at 28±2 ^{0}C for 24-48 h. Five drops of solution A (Appendix 11) and few drops of solution B (Appendix 11) was added to each tube and examined. Formation of red colour indicated nitrate reduction. Autoclaved fermentation broth (Appendix 12) supplemented with 5 g/l each of glucose, sucrose and mannitol was inoculated with test isolates and incubated at 28±2 ^{0}C for 24-48 h. Production of acid or acid with gas was recorded. Autoclaved starch agar (Appendix 13) plates were spot

inoculated with 10 µl of each isolate grown in broth and incubated at 28±2 ^{0}C for 24-48 h. After incubation, plates were flooded with iodine solution. Clear zone of hydrolysis around the bacterial growth indicated starch hydrolysis. Tubes containing autoclaved nutrient broth amended with 12% gelatin were inoculated with test isolates and incubated at 28±2 ^{0}C for 48 h. After incubation, tubes were kept at 4 ^{0}C for 30 min. On refrigeration, liquefied tubes indicated positive test for gelatin hydrolysis.

3.4.2 Identification based on 16s rDNA sequencing

Sequencing of 16S rDNA of the most promising bacterial strains namely, PS1, PS2, PS9 and PS19 recovered from mustard rhizosphere, demonstrating greater phosphate solubilizing activity *in vitro* was performed at Macrogen Inc., Seoul, South Korea using universal primers, 518F (5'CCAGCAGCCGCGGTAATACG3') and 800R (5'TACCAGGGTATCTAATCC3').

3.5 Agrochemicals used

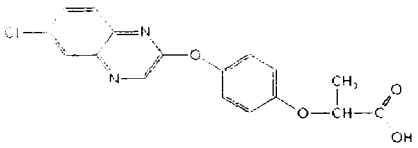
Technical and commercial formulations of three groups of pesticides including herbicides, like, quizalafop-p-ethyl, clodinafop, metribuzin, and glyphosate, insecticides, like, fipronil, pyriproxyfen, imidacloprid, and thiamethoxam and fungicides, like, tebuconazole, hexaconazole, metalaxyl and kitazin were used in this study. Pesticides and their dose rate applied in agricultural practices are shown in Table 11 and chemical structures of such pesticides are presented in Fig. 12.

3.6 Assessment of bacterial strains for pesticide tolerance

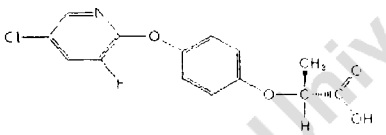
The bacterial strains were tested for their sensitivity/resistance to 12 pesticides, four each from three groups, by agar plate dilution method using minimal salt agar medium (Appendix 14). The freshly prepared agar plates amended separately with increasing concentration (0 to 3200 µg ml^{-1}; at a two fold dilution interval) of herbicides, insecticides and fungicides were spot inoculated with 10 µl of 10^8 cells ml^{-1} of bacterial strains. Each experiment was replicated three times. Plates were incubated at 28 ± 2°C for three days and the highest concentration of pesticides supporting bacterial growth was defined as the maximum resistance level (MRL).

11 Agrochemicals used in the present study

Common Name		Grade and Purity	Chemical Name	Chemical Family	Recommended Dose	Source
cides	Quizalafop-p-ethyl	Technical (98% w/w)	(*RS*)-2-[4-(6-chloroquinoxalin-2-yloxy)phenoxy]propionic acid	Aryloxyphenoxy	40 µg/kg	Parijat Agrochemicals, New Delhi, India
	Clodinafop	Technical (98%w/w)	(*R*)-2-[4-(5-chloro-3-fluoro-2-pyridyloxy)phenoxy]propionic acid	Aryloxyphenoxy	400 µg/kg	Parijat Agrochemicals, New Delhi, India
	Metribuzin	Commercial (70%w/w)	4-amino-6-*tert*-butyl-4,5-dihydro-3-methylthio-1,2,4-triazin-5-one	Triazinone	850 µg/kg	Singhal Pesticides, Mumbai, India
	Glyphosate	Commercial (71% w/w)	*N*-(phosphonomethyl)glycine	Organophosphorus	1444 µg/kg	Excel Crop Core LTD., Mumbai, India
icides	Fipronil	Technical (98%w/w)	5-amino-1-(2,6-dichloro-α,α,α-trifluoro-*p*-tolyl)-4-trifluoromethylsulfinylpyrazole-3-carbonitrile	Phenylpyrazole	200 µg/L	Parijat Agrochemicals, New Delhi, India
	Pyriproxyfen	Technical (98%w/w)	4-phenoxyphenyl (*RS*)-2-(2-pyridyloxy)propyl ether	Juvenile hormone mimics	1300 µg/L	Parijat Agrochemicals, New Delhi, India
	Imidacloprid	Technical (100% EC)	(*E*)-1-(6-chloro-3-pyridylmethyl)-*N*-nitroimidazolidin-2-ylideneamine	Pyridylmethylamine	100 µg/L	Parijat Agrochemicals, New Delhi, India
	Thiamethoxam	Technical (100%w/w)	(*EZ*)-3-(2-chloro-1,3-thiazol-5-ylmethyl)-5-methyl-1,3,5-oxadiazinan-4-ylidene(nitro)amine	Thiazole	25 µg/L	Parijat Agrochemicals, New Delhi, India
;ides	Tebuconazole	Technical (100%w/w)	(*RS*)-1-*p*-chlorophenyl-4,4-dimethyl-3-(1*H*-1,2,4-triazol-1-ylmethyl)pentan-3-ol	Conazole	100 µg/kg	Parijat Agrochemicals, New Delhi, India
	Hexaconazole	Technical (100%w/w)	(*RS*)-2-(2,4-dichlorophenyl)-1-(1*H*-1,2,4-triazol-1-yl)hexan-2-ol	Conazole	40 µg/kg	Parijat Agrochemicals, New Delhi, India
	Metalaxyl	Commercial (35%w/w)	methyl *N*-(methoxyacetyl)-*N*-(2,6-xylyl)-DL-alaninate	Anilide	1500 µg/kg	Tropical Agrosystem LTD., Chennai, India
	Kitazin	Commercial (48% EC)	O,O-Bis(1-methylethyl) S-phenylmethyl phosphorothioate	Organophosphate	96 µg/kg	P.I. Industries LTD., Rajasthan, India



Quizalafop-p-ethyl



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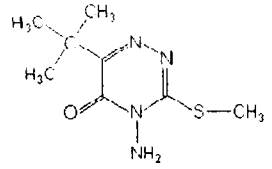

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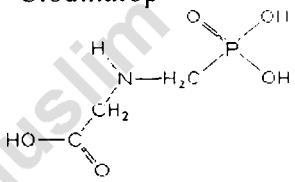
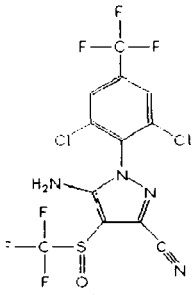


Glyphosate

Herbicides



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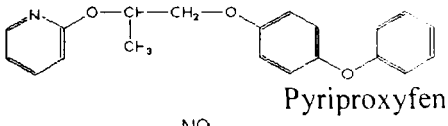
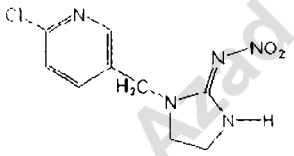
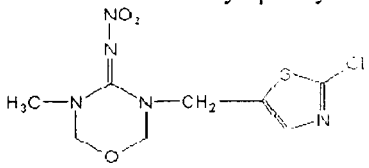


Pyriproxyfen

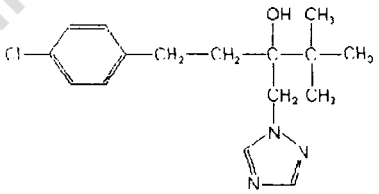


Imidacloprid

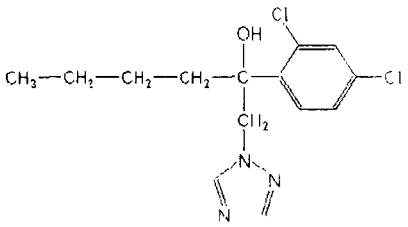


Thiamethoxam

Insecticides



Tebuconazole



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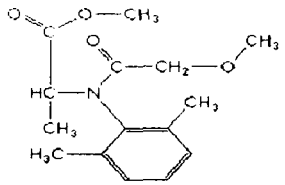
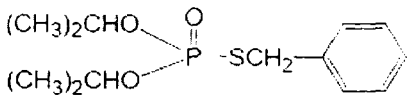


Metalaxyl



Kitazin

Fungicides

Fig. 12: Chemical structures of agrochemicals used in the present study

3.7 Bioassay of plant growth promoting activities under pesticide stress

Plant growth promoting (PGP) activity of rhizobacteria was assessed both in the presence and absence of the selected groups of pesticides under *in vitro* conditions. The plant growth promoting activities including indole acetic acid (IAA) production, phosphate solubilization, siderophore production, hydrogen cyanide and ammonia production were assayed.

3.7.1 Quantitative assay of indole acetic acid

Indole-3-acetic acid synthesized by bacterial strains was quantitatively evaluated by the method of Gordon and Weber, (1951), later modified by Brick et al., (1991). For this activity, the nitrogen fixing and phosphate solubilizing bacterial strains were grown in Luria Bertani (LB) broth (Appendix 15). Luria Bertani broth (100 ml) having fixed concentration of tryptophan (100 µg/ml) supplemented with recommended (X), two times (2X) and three times (3X) of recommended rate of each pesticide and without pesticide (control) was inoculated with one ml culture (10^8 cells/ml) of both nitrogen fixing and phosphate solubilizing bacterial isolates and was incubated for 24 h at 28 ± 2 ^{0}C with shaking at 125 rpm. After 24 h, a five mililitre of culture of each treatment was centrifuged (9,000 g) for 15 min. and an aliquot of two ml supernatant was mixed with 100 µl of orthophosphoric acid and four mililitre of Salkowsky reagent (2% 0.5M $FeCl_3$ in 35% per-chloric acid) and incubated at 28 ± 2°C in darkness for 1h. The absorbance of developed pink color was read at 530 nm. The IAA concentration in the supernatant was determined using a calibration curve of pure IAA as a standard (Brick et al., 1991). The experiment was repeated three times on different time intervals.

3.7.2 Qualitative and quantitative estimation of siderophores

The nitrogen fixing and phosphate solubilizing bacterial strains were further tested for siderophore production using Chrome Azurol S (CAS) agar medium (Appendix 16) following the method of Alexander and Zuberer, (1991). Chrome Azurol S agar plates supplemented with 0, X, 2X and 3X of herbicides, insecticides and fungicides were prepared separately and divided into equal sectors and spot inoculated with 10 µl of 10^8 cells/ml and incubated at 28± 2°C for five days. Development of yellow orange halo around the bacterial growth was considered as positive for siderophore synthesis. Each individual experiment was repeated three times. The production of siderophore by the test strains were further detected quantitatively using Modi medium (Appendix 17). Modi medium amended with 0, X, 2X and 3X of each group of pesticides, was inoculated with 10^8 cells ml^{-1} of bacterial cultures and incubated at 28± 2 °C for

five days. Catechol type phenolates were measured on ethyl acetate extracts of the culture supernatant using a modification of the ferric chloride-ferricyanide reagent of Hathway. Ethyl acetate extracts was prepared by extracting 20 ml of supernatant twice with an equal volume of solvent at pH 2. Hathway's reagent was prepared by adding one milliliter of 0.1 M ferric chloride in 0.1 N HCl to 100 ml of distilled water, and to this, was added one milliliter of 0.1 M potassium ferricyanide (Reeves et al., 1983). For the assay, one volume of the reagent was added to one volume of sample and absorbance was determined at 560 nm for salicylates with sodium salicylate as standard and at 700 nm for dihydroxy phenols with 2, 3- dihydroxy benzoic acid (DHBA) as standard.

3.7.3 Phosphate solubilization by test bacterial strains

The bacterial strains showing phosphate solubilizing activity during screening process were inoculated onto Pikovskaya plates supplemented with 0, X, 2X and 3X of each pesticide and incubated at 28 ± 2 ^{0}C for seven days and observed for halo formation. The colony forming a clear halo around the bacterial growth was considered phosphate solubilizer. The solubilization index (SI) of the phosphate solubilizing organism was calculated as

$$\textbf{Solubilization index (SI)} = \frac{(\text{zone size including colony diameter} - \text{colony diameter})}{\text{zone size including colony diameter}}$$

The clear halo around bacterial growth was measured and bacterial cultures were further used to determine the extent of phosphate solubilization in liquid Pikovskaya medium. For the quantitative measurement of P, 100 ml of Pikovskaya broth treated with 0, X, 2X and 3X of each pesticide, was inoculated with one mL of 10^8 cells/ml of each culture. The flasks were incubated for seven days with shaking at 120 rpm at 28 ± 2 ^{0}C. A 20 ml culture broth from each flask was removed and centrifuged (9000 g) for 30 min. and the amount of water soluble P released into the supernatant was estimated by the chlorostannous-reduced molybdophosphoric acid blue method (King, 1932; Jackson, 1967). To 10 ml of supernatant, 10 ml chloromolybdic acid (Appendix 18) and 5 drops of chlorostannous acid (Appendix 19) was added and volume was adjusted to 50 ml with distilled water. The absorbance of developing blue colour developed was read at 600 nm. The amount of phosphate solubilized was calculated using the calibration curve of KH_2PO_4. The change in pH following tri-calcium phosphate (TCP) solubilization was also recorded. Each independent experiment was repeated three times after several subcultures. The bacterial isolates showing greater solubilization on both solid and liquid medium and

maintaining the PS activity after several subcultures were chosen as the efficient PS strains for further studies.

3.7.4 Assay of hydrogen cyanide (HCN) and ammonia

Hydrogen cyanide production by bacterial isolates was detected by the method of Bakker and Schipper, (1987). For HCN production, all plant growth promoting rhizobacterial strains were grown on an HCN induction medium (Appendix 20) supplemented with normal, double and three times more of each pesticide at 28 ± 2 ^{0}C for four days. For each bacterial isolate, 100 µl of 10^8 cells/ml was placed in the centre of the petri plates. A disk of Whatman filter paper No. 1 dipped in 0.5% picric acid and 2% Na_2CO_3 was placed at the lid of the petri plates. Plates were sealed with parafilm. After four days incubation at 28 ± 2 ^{0}C, an orange brown colour of the paper indicating HCN production was observed. For ammonia assessment, the bacterial strains were grown in peptone water (Appendix 21) with 0, X, 2X and 3X of each pesticide and incubated at 28 ± 2 ^{0}C for four days. One mililitre of Nessler reagent (Appendix 22) was added to each tube and the development of yellow color indicating ammonia production was recorded following the method of Dye (1962).

3.7.5 Bioassay of exopolysaccharides

The exo-polysaccharides (EPS) produced by the bacterial strains was determined under *in vitro* conditions as suggested by Mody et al. (1989). For this, the bacterial strains were grown in 100 ml capacity flasks containing basal medium supplemented with 5% sucrose and treated with 0, X, 2X and 3X of each pesticide. Inoculated flasks were incubated for five days at 28±2 °C on rotary shaker (100 rpm). Culture broth was spun (5433 g) for 30 min. and EPS was extracted by adding three volumes of chilled acetone (CH_3COCH_3) to one volume of supernatant. The precipitated EPS was repeatedly washed three times alternately with distilled water and acetone, transferred to a filter paper and weighed after overnight drying at room temperature.

3.8 Pesticidal toxicity to legumes

The experiment was conducted to evaluate the toxic effects of recommended (X), double (2X) and three times more of recommended rates (3X) of pesticides on chickpea (var.C235), lentil (var. K75), greengram (var. K851), and pea (var. arakle), grown in clay pots.

3.8.1 Pesticide treatments and plant culture

Seeds of the commonly grown legumes like, chickpea, lentil and greengram were purchased from Prakash Agrochemicals and Seeds, Aligarh, U.P., India while seeds of pea were

obtained from Indian Agricultural Research Institute (IARI), Pusa, New Delhi, India. Seeds of chickpea, lentil, greengram, and pea were surface sterilized with 70% ethanol, 3 min.: 3% sodium hypochlorite, 3 min. rinsed six times with sterile water and dried. A total of 10 seeds were sown in clay pots (25 cm high, 22 cm internal diameter) using three kg unsterilized soils (Appendix 23) with control (without pesticides) and three treatments each with recommended dose (X), two times (2X) and three times of recommended (3X) dose rate of each pesticide (Table 12). Seeds of legumes were sown on October 15, 2006 (chickpea), November 7, 2006 (lentil), March 15, 2007 (greengram) and November 7, 2006 (pea). A total of six pots used for each treatment were arranged in a complete randomized design. Plants in each pot were thinned to three plants 10, 10, 7 and 7 days after emergence (DAE) of chickpea, lentil, greengram and pea, respectively. The pots were watered with tap water when required and were maintained in an open field. The experiments were conducted for two consecutive years with the identical environmental conditions and with the same pesticide treatments to ensure the reproducibility of the results.

Table 12 Pesticidal treatment of experimental soils

Pesticides applied		Dose rate (µg kg^{-1}soil)		
		Recommended(X)	Double (2X)	Triple (3X)
Herbicides	Quizalafop-p-ethyl	40	80	120
	Clodinafop	400	800	1200
Insecticides	Fipronil	200	400	600
	Pyriproxyfen	1300	2600	3900
Fungicides	Tebuconazole	100	200	300
Control (without pesticide)		-	-	-

3.8.2 Parameters measured

3.8.2.1 Biomass production and symbiotic attributes

All plants in three pots for each treatment were removed at 90 and 135 days after sowing (DAS) of chickpea, 90 and 120 DAS for pea and lentil and 50 and 80 DAS for greengram, respectively. The roots were carefully washed and nodules from the root systems of each legume were separated, counted, oven dried at 80 ^{0}C and weighed. Plant growth, such as length of roots and shoots, dry weights of root and shoot and total dry plant biomass of all the four legumes was recorded at each sampling dates. Plants uprooted at all the sampling intervals were oven dried at 80 ^{0}C to measure the total plant biomass. The leghaemoglobin (Lb) content in fresh nodules recovered from the root system of each legume crop grown under pesticide stress and without

pesticide (control) was quantified at 90 DAS each for chickpea and pea and lentil and 50 DAS for greengram, respectively, by the method of Sadasivam and Manickam, (1992). Fresh nodules were crushed with the help of mortar and pestle in 5 ml sodium phosphate buffer (pH 7.4) and filtered through two layers of cheese cloth. The nodule debris was discarded. The turbid reddish brown filtrate was clarified by centrifugation at 10000 g for 30 min. The supernatant was diluted to 10 ml with sodium phosphate buffer (pH 7.4) (Appendix 24). The extract was divided equally into two glass tubes (5 ml /tube) and equal amount of alkaline pyridine reagent (Appendix 25) was added to each tube. The haemochrome formed was read at 556 and 539 nm after adding a few crystals of potassium hexacyanoferrate and sodium dithionite, respectively. The leghaemoglobin content was calculated using the formula –

$$\text{Lb content (mM)} = \frac{[A_{556} - A_{535}] \times 2D}{23.4}$$

Where D = initial dilution

3.8.2.2 Total chlorophyll, nitrogen and phosphorus contents

The total chlorophyll content in fresh foliage of each experimental legume crop was quantified at 90 DAS each for chickpea, pea and lentil and 50 DAS for greengram by the method of Arnon, (1949). Briefly, one gram of fresh leaves of each legume was grinded in 40 ml of 80% acetone with the help of mortar and pestle. The suspension was decanted in Buchner funnel having Whatman filter paper No. 1. The residue was grounded three times with acetone and the resulting suspension was filtered again. Contents in mortar-pestle was washed with 80% acetone and filtered. The filtrate was transferred to 100 ml volumetric flask and volume was made upto 100 ml. The absorbance was read at 645 and 663 nm using double beam UV-Visual spectrophotometer (Electronics Corporation of India Limited, India). The total chlorophyll content was calculated as –

$$\text{Total chlorophyll} = \frac{[20.2\ (OD_{645}) + 8.02\ (OD_{663})] \times V}{1000 \times W}$$

Where OD_{645} = optical density at 645 nm,

OD_{663} = optical density at 663 nm

V = final volume of chlorophyll extract in 80% acetone and

W = fresh weight of tissue extracted

The total nitrogen and phosphorus content in roots and shoots of chickpea, lentil, pea and greengram were measured at harvest by the micro-Kjeldahl method of Iswaran and Marwah,

(1980) and the method of Jackson (1967), respectively. Briefly, 50 ml of the sample was taken in the Kjeldahl flask, moistened with 5 ml water, containing 15 ml N/100 ml H_2SO_4 and shaken thoroughly. This was followed by the addition of N $KMnO_4$ in small amount untill pink color appeared. The catalyst mixture (3 g K_2SO_4, 0.3 g $FeSO_4$. 5 H_2O and 0.15 g $CuSO_4$. $5H_2O$) was then added and sample was digested for 30 min. on low flame untill the mixture became yellowish green.

3.8.2.3 Seed yield and grain protein

Chickpea, pea, lentil and greengram were finally harvested at 135, 120, 120 and 80 DAS, respectively, and seed yield was measured. The protein content in grains of each legume was estimated by the method of Lowrey, (1951). For the protein estimation, 500 mg of seeds were soaked in phosphate buffer (pH 7.4) and grinded thinly in 5-10 ml phosphate buffer (pH 7.4) (Appendix 22). The extract was centrifuged (4000 rpm) and the supernatant was used for protein analysis. A 0.2 ml aliquot was taken from the sample extract and the volume was made upto one ml in each test tube, followed by addition of 5 ml copper solution (Appendix 26) to each test tube. Each sample was mixed well and allowed to stand for 10 min. and 0.5 ml Folins reagent (Appendix 27) was added to each test tube and incubated at room temperature for 30 min. Absorbance of blue colour was read at 660 nm. The protein concentration in the supernatant was determined using a calibration curve of bovine serum albumin (BSA) as a standard.

3.8.2.4 Statistical analysis

Each pot in this study was considered as a replicate and each individual treatment was replicated six times for chickpea, pea, lentil and greengram. Since the experiment was conducted consecutively for two years under the identical environmental conditions, data of the measured parameters were pooled together. The data were subjected to analysis of variance (ANOVA) and least significant difference (LSD) was calculated at 5% probability level.

3.8.3 Bioremediation studies using pesticide resistant plant growth promoting rhizobacterial strains

The rhizobial strains *Mesorhizobium* MRC4, *Rhizobium* MRP1, *Bradyrhizobium* MRM6 *Rhizobium* MRL3 and phosphate solubilizing bacterium *Pseudomonas aeruginosa* PS1 resistant to herbicides (quizalafop-p-ethyl, clodinafop, metribuzin, and glyphosate), insecticides (fipronil, pyriproxyfen, imidacloprid, and thiamethoxam) and fungicides (tebuconazole, hexaconazole, metalaxyl and kitazin) isolated in the present investigation were used to determine their

bioremediation potential using chickpea, pea, lentil and greengram as a test crop, when grown in the soil treated with or without herbicides, insecticides and fungicides.

3.8.3.1 Microbial treatments, pesticide application and legume growth

Prior to inoculation of seeds with PGPR, the cell suspension of isolate was grown in YEM broth (for rhizobia) and Pikovskaya broth (for phosphate solubilizer) in flasks shaken at 120 rpm at 28± 2 °C for five and three days respectively to a cell density of 6 x 10^8 (rhizobia) and 3 x 10^7 cells/ml (for phosphate solubilizers). Seeds of chickpea (var C235), lentil (var. K75), pea (var. arakle), and greengram (var. K 851) were surface sterilized (Vincent, 1970) and were coated separately with pesticide resistant plant growth promoting *Mesorhizobium* strain MRC4, *Rhizobium* strain MRL3, *Rhizobium* strain MRP1 and *Bradyrhizobium* strain MRM6, respectively. Of the phosphate solubilizers, only *Pseudomonas aeruginosa* PS1 was used to inoculate only greengram seeds. Seeds of each legume were soaked in liquid culture medium for 2 h using 10% gum arabic as sticker to deliver approximately 10^8 cells/seed for rhizobia and 10^{-7} cells/seed for *Pseudomonas aeruginosa* PS1. The non-coated sterilized seeds used as control were soaked in sterile water only. The non-inoculated and inoculated seeds (10 seeds per pot) were sown on October 15, 2006 (chickpea), November 7, 2006 (lentil), March 15, 2007 (greengram) and November 7, 2006 (pea) in clay pots (25 cm high, 22 cm internal diameter) using three kg unsterilized sandy clay loam soil (Appendix 23) treated with or without pesticides. The normal concentrations of herbicides, insecticides and fungicides used in this study were similar to those used for phytotoxicity evaluation against each legume (Table 12). Each treatment was replicated six times for all crops under study and was arranged in a completely randomized design. Plants in each pot were thinned to three plants 10, 10, 7 and 7 days after emergence (DAE) of chickpea, lentil, greengram and pea, respectively. The pots were watered with tap water when required and were maintained in open field conditions. All treatments were repeated the following year with the identical environmental conditions to ensure the reproducibility of the results.

Three pots having three plants per pot for each treatment were removed at 90 and 135 days after seeding (DAS) for chickpea, 50 and 80 DAS for greengram, and 90 and 120 DAS for pea and lentil, respectively. Plant growth such as the length of roots and shoots and dry matter accumulation in roots, shoots and whole plants was recorded at each sampling intervals. The remaining three pots for each treatment, having three plants per pot were maintained until

harvest. The total N and P content in roots and shoots for all the legume crops were measured at each sampling day by the micro-Kjeldahl method (Iswaran and Marwah, 1980) and the method of Jackson (1967), respectively. The total chlorophyll contents in fresh foliage of each legumes grown in metal stressed soil was quantified at 90 DAS for chickpea, pea and lentil and 50 DAS (greengram) by the method as discussed earlier. The leghaemoglobin content in fresh nodules recovered from the root system of each legume crop raised under pesticide stressed and pesticide free soils (control) was quantified at 90 DAS for chickpea, pea and lentil and 50 DAS for greengram, respectively, by the method as discussed earlier. Seed yield and grain protein (Lowrey, 1951) in chickpea, pea, lentil and greengram were estimated at harvest. Data of the measured parameters recorded for two years were pooled together and subjected to analysis of variance (ANOVA) for two factor (inoculation and pesticide concentration) pot culture experiment and significant partial difference (LSD) was calculated at 5% probability level.

Results

4.1 Microbial diversity in different rhizospheric soils

The rhizospheric soils of chickpea, greengram, lentil, pea and mustard grown at the experimental fields of Faculty of Agricultural Sciences, A.M.U., Aligarh, were subjected to microbial analysis (Table 13). The viable counts of bacteria, fungi and phosphate solubilizing microorganisms (PSM) differed considerably among rhizosphere soils. Generally, the microbial populations were highest in mustard rhizosphere soils compared to other soil samples. The bacterial populations in the rhizosphere of chickpea, greengram, lentil and pea were 3.2×10^7, 2.9×10^7, 3.5×10^7 and 3.1×10^7 CFU/g soil, respectively. In contrast, the rhizospheric soils of mustard showed a substantial increase of 36, 52, 23 and 29 percent in bacterial populations compared to those recovered from chickpea, greengram, lentil, and pea, respectively. The fungal populations in all the rhizospheric soils ranged from 1.2×10^5 (lentil) to 2.1×10^5 (greengram) CFU/g soil. In general, the populations of phosphate solubilizing bacteria (PSB) were comparatively more than the phosphate solubilizing fungi (PSF) in all soil samples. Among all the rhizosphere soils, the populations of PSB were greater in the rhizosphere of both chickpea and mustard and PSF counts were highest in both pea and mustard rhizosphere. However, No PSF was recovered from chickpea rhizosphere (Table 13).

4.2 Characterization of nitrogen fixing and phosphate solubilizing bacteria

In the present study, a total of 50 strains each belonging to genera *Mesorhizobium*, *Bradyrhizobium* and *Rhizobium* were isolated from the nodules of chickpea, greengram, lentil and pea crops using yeast extract mannitol agar plates. In addition, 50 strains of PSB were also isolated from the rhizospheric soils of mustard. Among the bacterial strains, 22% of *Mesorhizobium* spp. (chickpea), 18% of *Bradyrhizobium* spp. (greengram), 14% of *Rhizobium* spp. (pea), 16% of *Rhizobium* spp. (lentil) and 36% of PSB were selected for assaying the plant growth promoting activities. The isolated bacterial cultures showed a variable morphological and biochemical characteristics (Table 14). Generally, the rhizobial strains were Gram negative while PSB showed a variable Gram reaction. Rhizobial strains in general were positive to all the biochemical reactions except methyl red, Voges Proskauer, indole and gelatin hydrolysis test. In contrast, the PSB (Plate 1A-1H) showed a considerable variability in biochemical properties.

4.2.1 Identification of selected PGPR strains by 16s rDNA sequencing

On the basis of cultural, morphological and biochemical characteristics and comparing such properties with those given in Bergey's Manual of Determinative Bacteriology, the plant

growth promoting rhizobacteria isolated from host specific nodules of legume plants were tentatively grouped as *Mesorhizobium* spp. (chickpea), *Rhizobium* spp. (pea), *Rhizobium* spp. (lentil) and *Bradyrhizobium* spp. (greengram-vigna). While other PGPR belonged to genera *Bacillus*, *Pseudomonas*, *Enterobacter* and *Klebsiella*. Moreover, of the 50 phosphate solubilizing isolates, four isolates namely, PS1 (Plate 1A), PS2 (Plate 1B), PS9 (Plate 1E) and PS19 (Plate 1G) showing highest degree of TCP solubilization were selected for genetic characterization. Based on 16s rDNA gene analysis performed commercially at Macrogen Inc., Seoul, South Korea, these isolates were identified as *Pseudomonas aeruginosa* (Gene Bank accession number FJ705886), *Enterobacter asburiae* (Gene Bank accession number FJ705887), *Pseudomonas putida* (Gene Bank accession number FJ705888) and *Klebsiella sp.* (Gene Bank accession number FJ705889), respectively.

4.3 Functional diversity among plant growth promoting rhizobacteria

Of the total 200 nitrogen fixers and 50 phosphate solubilizers, a total of 35 nitrogen fixers and 18 phosphate solubilizers were screened for their multiple plant growth promoting (PGP) traits. Based on the PGP activity expressed by the bacterial strains under *in vitro* conditions, the mesorhizobial strains were grouped into four PGP groups (Table 15). The PGP group I included four strains which showed five PGP traits like production of ammonia, hydrogen cyanide, siderophore, indole acetic acid and exo-polysaccharides followed by PGP group II, which had three strains positive to ammonia, HCN and IAA. In PGP group III, three strains exhibited a positive reaction to ammonia and IAA, while PGP group IV included only one strain (MRC14) which showed only the synthesis of indole acetic acid. Similarly, *Rhizobium* strains isolated from pea nodules were grouped into three PGP groups (Table 16). The PGP group I had three isolates and displayed five PGP traits (i.e. production of ammonia, HCN, siderophore, IAA and exo-polysaccharides). This was followed by group II, which had only one strain (MRP2) and was positive for HCN and IAA. The group III contained three strains which showed positive reactions for ammonia, HCN and IAA. *Bradyrhizobium* strains were grouped into three PGP groups (Table 17). In PGP group I, 33% strains displayed five PGP traits like synthesis of ammonia, HCN, siderophore, IAA and exopolysaccharides. This was followed by group II which incorporated five strains, positive for three PGP traits, ammonia, siderophores and indole acetic acid. While, PGP group III was represented by only one strain (MRM4) that produced ammonia and IAA. *Rhizobium* strains isolated from lentil nodules were grouped into four PGP groups

(Table 18). The PGP group I comprised of three isolates and expressed five PGP traits (ammonia, HCN, siderophore, IAA and exo-polysaccharides) while the group II included only one strain (*Rhizobium* MRL7) which was positive for ammonia, siderophore and indole acetic acid. The group III had three strains which were positive to ammonia, hydrogen cyanide and IAA. The group IV exhibited one strain that was positive for ammonia and IAA. Similarly, PSB strains were grouped into four PGP groups (Table 19). The PGP group I represented four (22%) isolates with six PGP traits (ammonia, HCN, siderophore, IAA, phosphate solubilization and exopolysaccharides) whereas group II had only six strains (33%) which were positive for ammonia, siderophore, IAA, phosphate solubilization and exo-polysaccharides. The group III contained 28% of the strains which were found to be positive for ammonia, hydrogen cyanide, IAA, phosphate solubilization and exopolysaccharides. The group IV included three (17%) of the strains which were positive for ammonia, IAA, phosphate solubilization and exopolysaccharides.

4.4 Tolerance of plant growth promoting rhizobacteria to pesticides

The PGPR strains showing greatest plant growth promoting activities *in vitro* were selected to evaluate the effects of varying concentrations of herbicides, like, quizalafop-p-ethyl, clodinafop, glyphosate and metribuzin; insecticides, fipronil, pyriproxyfen, imidacloprid and thiamethoxam and fungicides, tebuconazole, hexaconazole, metalaxyl and kitazin using agar plate dilution method. Generally, the PGPR strains showed a varied level of tolerance to different chemical groups of pesticides added to minimal salts agar medium. Among the *Mesorhizobium* strains, strain MRC4 showed the highest tolerance to most of the pesticides. Strain MRC4 tolerated a concentration of 1600, 1800, 3200 and 3000 µg/ml of quizalafop-p-ethyl, clodinafop, metribuzin and glyphosate, respectively (Fig. 13) while it also showed a tolerance level of 1600, 1400, 2400 and 2800 µg/ml to fipronil, pyriproxyfen, imidacloprid and thiamethoxam, respectively (Fig. 14), and 1600, 2200, 2800 and 3200 µg/ml to tebuconazole, hexaconazole, metalaxyl and kitazin, respectively (Fig. 15). In contrast, strain MRP1 (Fig. 16–18), MRM6 (Fig. 19–21) and MRL3 (Fig. 22–24) of *Rhizobium* spp. (pea), *Bradyrhizobium* spp. and *Rhizobium* spp. (lentil), respectively, showed highest tolerance to most of the tested pesticides. Among the rhizobacterial strains, strain MRP1 exhibited a higher tolerance to quizalafop-p-ethyl (1600 µg/ml), clodinafop (2400 µg/ml), metribuzin (3000 µg/ml), glyphosate (2800 µg/ml), fipronil (2800 µg/ml), pyriproxyfen (1800 µg/ml), imidacloprid (1600 µg/ml), thiamethoxam (2200 µg/ml), tebuconazole (1400 µg/ml), hexaconazole (2000 µg/ml), metalaxyl (2600 µg/ml) and

kitazin (3000 µg/ml). Among a total of nine strains of *Bradyrhizobium* recovered from greengram nodules, strain MRM6 tolerated quizalafop-p-ethyl, clodinafop, metribuzin, glyphosate, fipronil, pyriproxyfen, imidacloprid, thiamethoxam, tebuconazole, hexaconazole, metalaxyl and kitazin to a level of 1600, 1600, 3200, 3000, 1600, 1600, 2200, 2400, 1600, 1800, 2800 and 3200 µg/ml, respectively (Fig. 19 – 21), while strain MRL3 showed a higher tolerance of 1600 µg/ml to quizalafop-p-ethyl, 1600 µg/ml to clodinafop, 3200 µg/ml to metribuzin, 2800 µg/ml to glyphosate, 1400 µg/ml to fipronil, 1600 µg/ml to pyriproxyfen, 2200 µg/ml to imidacloprid, 2400 µg/ml to thiamethoxam, 1600 µg/ml to tebuconazole, 2000 µg/ml to hexaconazole, 2600 µg/ml to metalaxyl and 3200 µg/ml to kitazin (Fig. 22–24). In contrast, of the 18 PSB, *Pseudomonas aeruginosa* PS1, *Enterobacter asburiae* PS2, *Pseudomonas putida* PS9 and *Klebsiella* sp. PS19, tolerated most of the tested herbicides (Fig. 25), insecticides (Fig. 26) and fungicides (Fig. 27). Of all the PGPR strains, *Pseudomonas aeruginosa* PS1 was the most tolerant bacterium which had a greater MRL values for all the herbicides, insecticides and fungicides. In the following studies, growth pattern of the pesticide tolerant strains, *Mesorhizobium* strain MRC4, *Rhizobium* strain MRP1, *Bradyrhizobium* strain MRM6, *Rhizobium* isolate MRL3 and *Pseudomonas aeruginosa* PS1 grown in minimal medium supplemented with different concentrations of 12 pesticides at different incubation intervals are presented in Fig. 28–42. Generally, the growth of PGPR strains was slow and increased somewhat linearly with increasing incubation intervals and after reaching a specific optical density, falls sharply. Among all pesticides, herbicide quizalafop-p-ethyl, insecticide pyriproxyfen and fungicide tebuconazole were found to be most toxic to bacterial growth.

4.5 Bioassay of plant growth promoting activities

The plant growth promoting substances like IAA, phosphate solubilization, siderophore, HCN and ammonia synthesized by the PGPR strains were determined both qualitatively and quantitatively under *in vitro* conditions.

4.5.1 Bioassay of Indole acetic acid

The production of IAA by the selected bacterial strains namely, *Mesorhizobium* spp. (N=11), *Rhizobium* spp. (pea, N=7), *Bradyrhizobium* spp. (N=9), *Rhizobium* spp. (lentil, N=8) and PSB (N=18) was assayed in LB broth supplemented with a concentration of (100 µg/ml) tryptophan. The *Mesorhizobium* spp. exhibited a substantial production of IAA after five days incubation. Moreover, a wide range of variability in the secreted amount of IAA was observed

among rhizobial isolates (Table 20). Of the mesorhizobial strains, strain MRC4 produced a maximum amount (44 µg/ml) of IAA and was followed by strain MRC5, which produced 43 µg IAA/ml in LB broth supplemented with 100 µg tryptophan/ml. Generally, the amount of IAA synthesized by mesorhizobial strains varied between 14 (MRC10) to 44 µg /ml (MRC4). The percent increase in IAA synthesized by MRC4 over other mesorhizobial strains ranged between 2 (MRC5) to 68 (MRC10) (Table 20). Among the pea specific *Rhizobium* isolates, strain MRP1 produced a detectable amount (32 µg/ml) of IAA in LB broth. This was followed by strain MRP3 which produced 28 µg IAA/ml in LB broth. In general, the amount of IAA synthesized by rhizobial strains varied between 17 (MRP4) to 32 µg /ml (MRP1). The percent increase in IAA synthesized by MRP1 over other rhizobial strains ranged between 12 (MRP3) to 47 (MRP4) (Table 21). *Bradyrhizobium* strains also produced a significant amount of IAA, maximum being 38 µg/ml IAA by the strain MRM6 followed by MRM5 (34 µg/ml). Indole acetic acid synthesized by bradyrhizobial strains varied between 15 (MRM7) to 38 µg /ml (MRM6). The increase in IAA synthesized by MRM6 over other rhizobial strains ranged between 11% (MRM5) to 61% (MRM7) (Table 22). In comparison, *Rhizobium* strains isolated from lentil nodules showed a substantial production of IAA. For instance, strain MRL3 secreted highest amounts (37 µg/ml) of IAA and was followed by strain MRL5 that produced 30 µg/ml IAA. Generally, IAA production by rhizobial strains varied between 15 (MRL2, MRL7) to 37 µg /ml (MRL3). The increase in IAA secreted by MRL3 relative to other rhizobial strains ranged between 19% (MRL5) to 59% (MRL2, MRL7) (Table 23). Similarly, the IAA production by phosphate solubilizing bacteria (N=18) was also assayed in this study (Table 24). Of these, *Klebsiella* sp. PS19 was most effective and produced a highest amount of IAA (42 µg/ml) followed by *Pseudomonas aeruginosa* PS1 (39 µg/ml), *Pseudomonas putida* PS9 (34 µg/ml) and *Enterobacter asburiae* PS2 (32 µg/ml) under normal conditions. Summarily, IAA synthesis by PSB strains varied between 9 (*Bacillus* sp. PS23) to 42 µg/ml (*Klebsiella* sp. PS19). The percent enhancement in IAA synthesis by *Klebsiella* sp. PS19 over other phosphate solubilizer ranged between 7 (*Pseudomonas aeruginosa* PS1) to 79 (*Bacillus* sp. PS23).

4.5.2 Bioassay of siderophores

In the present investigation, the production of siderophores was assayed qualitatively as well as quantitatively using CAS agar and ethyl acetate extraction method. On CAS agar plates, a total of 36% of the *Mesorhizobium* strains (Plate 2A) produced siderophore. The siderophore

detectable by the formation of orange yellow halo on CAS agar plates after five days of incubation varied between 9 (MRC10) to 12 mm (MRC4). Further, the ethyl acetate extraction from culture supernatant of *Mesorhizobium* strain MRC1 yielded 30 and 17 µg/ml salicylate (SA) and 2,3-dihydroxy benzoic acid (DHBA), strain MRC4 produced 35 and 19 µg/ml of SA and DHBA, strain MRC7 yielded 25 and 18 µg/ml SA and DHBA, and strain MRC10 yielded 21 and 17 µg/ml SA and DHBA, respectively. Among all the siderophore producing mesorhizobial strains, strain MRC4 displayed a substantial increase in SA (34%) and DHBA (12%) over the lowest siderophore producing bacterial strain (MRC10) (Table 20). Among the *Rhizobium* species isolated from pea nodules, only three (43%) strains were positive for siderophore activity where strain MRP1, MRP4 and MRP7 demonstrated 11, 10 and 11 mm orange yellow colored zone on CAS plates after five days of incubation (Table 21). Further, these strains produced 32 and 22 (strain MRP1), 29 and 18 (MRP4) and 25 and 14 (MRP7) µg/ml SA and DHBA, respectively. Of all the siderophore producing rhizobial strains, strain MRP1 showed a considerable increase in SA (22%) and DHBA (36%) compared to the lowest siderophore producing rhizobial strain (MRP7) (Table 21). Strains MRM3, MRM6 and MRM8 of *Bradyrhizobium* species showed 10, 13 and 11 mm orange yellow colored zone, respectively, on CAS agar plates after five days of incubation and produced 30 and 15 (strain MRM3), 32 and 18 (MRM6) and 28 and 16 (MRM8) µg/ml SA and DHBA, respectively. Among all the siderophore producing bradyrhizobial strains, strain MRM6 displayed a substantial augmentation in SA (13%) and DHBA (11%) relative to the lowest siderophore producing strain MRM8 (Table 22). Furthermore, among the *Rhizobium* species isolated from lentil nodules, 50% of the rhizobial isolates showed a positive reaction to siderophore both on CAS agar plates and in liquid culture medium. The siderophore halo size produced by such strains ranged between 10 (strain MRL1, MRL3, MRL7) to 12 mm (MRL6) and yielded 26 and 18 (MRL1), 29 and 21 (MRL3), 27 and 17 (MRL6) and 25 and 15 µg/ml SA and DHBA, respectively. Among all the siderophore producing rhizobial strains, strain MRL3 displayed a substantial increase in SA (14%) and DHBA (29%) over the lowest siderophore producing rhizobial strain (MRL7) (Table 23). The phosphate solubilizing bacteria (*Pseudomonas*, *Bacillus*, *Enterobacter* and Klebsiella) were also analyzed for siderophore production (Table 24). A total of 55% strains of selected phosphate solubilizing bacteria displayed the siderophore activity on CAS agar plates and also in liquid culture medium. Among the phosphate solubilizers, *Pseudomonas aeruginosa* PS1 (Plate 2B), *Enterobacter*

asburiae PS2, *Pseudomonas putida* PS9 and *Klebsiella* sp. PS19 had 15, 13, 14 and 14 mm colored zone, respectively on CAS plates. In liquid culture medium, *Pseudomonas aeruginosa* PS1 showed 41 and 21 µg/ml of SA and DHBA production. *Enterobacter asburiae* PS2 produced 24 and 9, *Pseudomonas putida* PS9 produced 41 and 17 and *Klebsiella* sp. PS19 produced 47 and 10 µg/ml of SA and DHBA, respectively. Of the siderophore producing phosphate solubilizing bacterial strains, *Pseudomonas aeruginosa* PS1 displayed considerable increase in SA (73%) and DHBA (76%) compared to the lowest siderophore producing bacterial strain (*Bacillus* sp. PS5) (Table 24).

4.5.3 Bioassay of exo-polysaccharides

The exo-polysaccharides (EPS) secretion by the pesticide tolerant bacterial strains was determined in culture supernatant (Table 20-24). The mesorhizobial strains substantially produced EPS after 120 h incubation. Generally, the amount of EPS released by rhizobacteria varied considerably among bacterial species. Among the bacterial strains, a total of 36, 43, 33, 38 and 100% of *Mesorhizobium*, *Rhizobium* (pea), *Bradyrhizobium*, *Rhizobium* (lentil) and PSB respectively, produced EPS. Of the mesorhizobial strains, strain MRC4 produced a maximum amount (21 µg/ml) of EPS and was followed by strain MRC1 (16 µg/ml) (Table 20). Among *Rhizobium* strains isolated from pea nodules, only three isolates secreted EPS in the culture medium wherein strain MRP1 produced a maximum amount (20 µg/ml) of EPS. However, strain MRP4 exhibited the lowest production of EPS (15 µg/ml) in broth (Table 21). *Bradyrhizobium* strains also produced a significant amount of EPS, maximum being 21 µg/ml EPS observed for the strain MRM6 followed by MRM8 which produced 14 µg/ml EPS (Table 22). *Rhizobium* strains specific to lentil plants also produced adequate amount of EPS. For example, strain MRL3 synthesized highest amount (18 µg/ml) of EPS which was followed by strain MRL1 (13 µg/ml) (Table 23). The EPS production by phosphate solubilizing bacteria ranged between 7 (*Bacillus* PS7 and *Bacillus* PS17) to 18 µg/ml (*Pseudomonas aeruginosa* PS1 and *Klebsiella* sp. PS19) (Table 24). Among all phosphate solubilizers, both *Pseudomonas aeruginosa* PS1 and *Klebsiella* sp. PS19 produced maximum amount of EPS (18 µg/ml) followed by *Pseudomonas putida* PS9 and *Enterobacter asburiae* PS2 that produced 17 µg/ml and 16 µg/ml EPS respectively.

4.5.4 Qualitative and quantitative assay of phosphorus

The plant growth promoting rhizobacteria were further evaluated for phosphate solubilizing (PS) potential, both on solid and in liquid Pikovskaya medium supplemented with 5 g/l tri-calcium phosphate (TCP). In the present study, a total of 34% rhizobacterial strains showed PS activity and formed a clear halo around bacterial growth. Generally, the size of phosphate solubilizing zone (halo) on solid Pikovskaya ranged from 4 (*Bacillus* sp. PS4) to 14 mm (*Klebsiella* sp. PS19). Among the phosphate solubilizing PGPR strains, *Pseudomonas aeruginosa* PS1, *Enterobacter asburiae* PS2, *Bacillus* sp. PS3, *Pseudomonas putida* PS9 and *Klebsiella* sp. PS19 displayed the largest zone of P solubilization on solid Pikovskaya medium after seven days of incubation. The percent increase in PS zone by the highest P activity showing *Klebsiella* sp. strain PS19 over other bacterial strains varied between 14% (*Pseudomonas aeruginosa* PS1, *Enterobacter asburiae* PS2 and *Bacillus* sp. PS3) to 71% (*Bacillus* sp. PS4) (Fig. 43). The solubilization index (SI) calculated based on size of bacterial colony and zone diameter ranged between 0.5 (*Bacillus* PS 4) to 2.5 (*Klebsiella* PS 19) (Fig. 45). Similarly, a considerable amount of tri-calcium phosphate (TCP) was solubilized in liquid culture by *Pseudomonas aeruginosa* PS1 (345 µg/ml), *Enterobacter asburiae* PS2 (258 µg/ml), *Pseudomonas putida* PS9 (298 µg/ml) and *Klebsiella* sp. PS19 (294 µg/ml). The percent increase in P solubilizing activity of *Pseudomonas aeruginosa* strain PS1, solubilizing maximum TCP in broth over other bacterial strains varied between 14% (*Pseudomonas putida* PS9) to 82% (*Bacillus* sp. PS23) (Fig. 44). The solubilization of TCP was accompanied by decrease in pH of the medium and a maximum decrease (35%) in pH was observed for strain PS9 compared to those observed for uninoculated control. The decrease in pH of the medium varied from strain to strain (Fig. 46) and generally, no strict relationship between amounts of solubilized P and change in pH was observed.

4.5.5 *In vitro* assay of ammonia and HCN

The plant growth promoting rhizobacterial strains were tested further for the synthesis of ammonia and hydrogen cyanide using peptone water and HCN induction medium, respectively. All growth promoting rhizobacterial strains of *Bradyrhizobium*, *Rhizobium* (lentil) and phosphate solubilizers showed a positive reaction for ammonia. In contrast, only 91% of *Mesorhizobium* and 86% of *Rhizobium* (pea) were positive for ammonia production as shown in Table 20–24. Furthermore, a total of 63% *Mesorhizobium*, 100% *Rhizobium* (pea) (Plate 2C), 33%

Bradyrhizobium, 75% *Rhizobium* (lentil) and 50% phosphate solubilizing strains were found to be positive for hydrogen cyanide production (Table 20–24).

4.6 Plant growth promoting activities under pesticide stress

A total of eight rhizobacterial strains including one strain each from *Mesorhizobium* spp., *Rhizobium* spp. (pea), *Bradyrhizobium* spp., *Rhizobium* spp. (lentil) and four phosphate solubilizers showing greater tolerance to pesticides under *in vitro* conditions were evaluated further for plant growth promoting activities in their respective medium supplemented with selected pesticides at recommended and higher dose rates. Among the PGPR strains, *Mesorhizobium* strain MRC4, *Rhizobium* strain MRP1 (pea), *Bradyrhizobium* strain MRM6, *Rhizobium* strain MRL3 (lentil), *Pseudomonas aeruginosa* strain PS1, *Enterobacter asburiae* strain PS2, *Pseudomonas putida* strain PS9 and *Klebsiella sp.* strain PS19 were chosen to evaluate the PGP potentials under herbicides, insecticides and fungicides stress due to their ability to express the greatest production of PGP substances when grown in medium devoid of pesticides.

4.6.1 Indole acetic acid production under pesticides stress

In this study, the effect of three concentrations (recommended dose – X, double of recommended dose – 2X and three times more of recommended dose – 3X) of herbicides (quizalafop-p-ethyl, clodinafop, metribuzin and glyphosate), insecticides (fipronil, pyriproxyfen, imidacloprid and thiamethoxam) and fungicides (tebuconazole, hexaconazole, metalaxyl and kitazin) on IAA synthesized by *Mesorhizobium* spp. was determined in LB broth treated with 100 µg/ml of tryptophan (Table 25). Most promising tolerant strain to all pesticides, *Mesorhizobium* sp. MRC4 produced a considerable amount of IAA both in the absence and presence of herbicides, insecticides and fungicides (Table 25). In the medium devoid of any pesticide, *Mesorhizobium* strain MRC4 produced maximum (44 µg/ml) of IAA. In contrast, the amount of IAA released by the mesorhizobial strain however, decreased progressively with increase in concentrations of herbicides, insecticides and fungicides. In case of herbicides, metribuzin had the least toxic effect on IAA synthesis while quizalafop-p-ethyl decreased IAA production most prominently by mesorhizobial strain MRC4. Of all the herbicides, metribuzin reduced the IAA production by 7, 13 and 16% while quizalafop-p-ethyl by 57, 66 and 75% at X, 2X and 3X concentration, respectively, over untreated control. Among insecticides, pyriproxyfen affected IAA production most severely and decreased it by 35, 53 and 62% and fungicide

tebuconazole decreased it by 62, 69 and 75% at X, 2X and 3X, respectively, relative to control (Table 25). *Rhizobium* strain MRP1 specific to pea when grown in LB medium devoid of pesticides produced 32 µg/ml IAA. However, under pesticide stress, IAA production decreased consistently as the concentration of herbicides, insecticides and fungicides was increased from normal to three times more concentration of each pesticide. Among herbicides, quizalafop-p-ethyl displayed most toxic effect on IAA production and decreased it upto 44% at 3X over control. Interestingly, decrease in IAA synthesis by both clodinafop and metribuzin was comparable. Among insecticides, fipronil showed highest toxicity and decreased IAA production by 22, 25 and 35% at X, 2X and 3X respectively in comparison to control. Thiamethoxam (insecticide) mediated effect on IAA production was not pronounced as observed for fipronil, pyriproxyfen and imidacloprid. Of the four fungicides, tebuconazole showed the greatest toxicity on IAA and decreased it by 32, 47 and 50% at X, 2X and 3X, respectively, compared to control. In contrast, kitazin had the least toxic effect on IAA synthesis (Table 26).

Among the *Bradyrhizobium* strains, strain MRM6 displayed a maximum of 38 µg/ml IAA with 100 µg/ml tryptophan but devoid of any pesticide. The effect of three concentrations each of herbicides, insecticides and fungicides on IAA production however, differed considerably (Table 27). The strain MRM6 when grown in LB medium amended with normal rates of quizalafop-p-ethyl, clodinafop, metribuzin and glyphosate produced 7, 17, 30 and 28 µg/ml IAA which significantly declined by 8, 18, 32 and 40% respectively, at 3X of all herbicides over control. Among three concentrations of each herbicide, the 3X of quizalafop-p-ethyl was most toxic and reduced the production of IAA by 57% compared to those observed for normal rate of the same herbicide. A recommended rate of fipronil, pyriproxyfen, imidacloprid and thiamethoxam strain MRM6 produced 27, 16, 32 and 35 µg/ml IAA, respectively. Indole acetic acid synthesis decreased progressively on increasing the concentrations of each insecticide but fipronil and pyriproxyfen showed highest toxicity and decreased the amount of IAA production by 69% and 82% respectively, compared to control. Among three concentrations of each insecticide, the 3X of both fipronil and pyriproxyfen showed the most toxic effect and reduced the IAA biosynthesis by 56% relative to those observed for recommended rates of the same insecticides. Further, strain MRM6 when used with normal dose of tebuconazole, hexaconazole, metalaxyl and kitazin, produced 7, 28, 35 and 36 µg/ml IAA, respectively. Like the effect of 3X of both herbicides and insecticides, the 3X of fungicides in general, had greatest

toxic effect on IAA synthesis by MRM6; the maximum being observed for 3X of tebuconazole which reduced IAA by 89% over untreated control. Of the three concentrations of each fungicide, the three times of recommended dose rate of tebuconazole showed the greatest toxicity and reduced the IAA synthesis by 43% relative to those observed for recommended rate of the same fungicide (Table 27).

Rhizobium strain MRL3 specific to lentil produced normally, 37 µg/ml IAA without pesticides stress. However, in presence of different concentrations of herbicides, insecticides and fungicides, IAA production by *Rhizobium* strain MRL3 decreased progressively as the concentration of each pesticide was increased gradually. Both quizalafop-p-ethyl and clodinafop most adversely affected IAA production and decreased it by 45% and 41% at 3X concentration when compared with untreated control. Metribuzin and glyphosate, though also inhibited the IAA synthesis but the effect was less pronounced. Among three concentrations of each herbicide, the three times of recommended dose rate of clodinafop was most toxic and reduced the IAA production by 33% relative to those observed for recommended rate of the same herbicide. When grown with the normal concentrations of fipronil, pyriproxyfen, imidacloprid and thiamethoxam treatment, strain MRL3 produced 31, 28, 26 and 28 µg/ml IAA respectively, after five days of incubation. However, pyriproxyfen showed the highest toxicity and decreased IAA production by 46% at 3X over control. Of three concentrations of each insecticide, 3X of pyriproxyfen displayed the most inhibitory effect and decreased the synthesis of IAA by 29% over those observed for normal rate of the same insecticide. Among fungicides, tebuconazole gave the highest inhibitory effect and decreased IAA by 59% at three times of recommended dose. Among three concentrations of each fungicide, the three times of normal rate of tebuconazole was most toxic and reduced the IAA production by 29% relative to those observed for normal rate of the same fungicide. Though the effect of other fungicides was also negative for IAA but to a lesser extent in comparison to tebuconazole (Table 28).

Phosphate solubilizing bacteria namely, *Pseudomonas aeruginosa* PS1, *Enterobacter asburiae* PS2, *Pseudomonas putida* PS9 and *Klebsiella* sp. PS19 tolerant to herbicides, insecticides and fungicides were also tested for IAA production under pesticide stressed environment (Table 29–32). Generally, the synthesis of IAA by the P-solubilizers decreased consistently with increasing concentrations of herbicides, insecticides and fungicides. However, production of IAA by the four selected pesticide tolerant and P solubilizing strains

(*Pseudomonas aeruginosa* PS1, *Enterobacter asburiae* PS2, *Pseudomonas putida* PS9 and *Klebsiella* sp. PS19) under pesticides stress conditions did not differ significantly. In general, *Pseudomonas aeruginosa* PS1, *Enterobacter asburiae* PS2, *Pseudomonas putida* PS9 and *Klebsiella* sp. PS19 produced 39, 32, 34 and 42 µg/ml IAA when grown in LB broth lacking pesticides. While, the biotoxicity of quizalafop-p-ethyl among herbicides, pyriproxyfen within insecticides and tebuconazole in fungicides group was most prominent over bacterial IAA biosynthesis. Quizalafop-p-ethyl decreased the synthesis of IAA by 90, 91, 88 and 84%; pyriproxyfen by 85, 72, 80 and 79% and tebuconazole by 92, 94, 95 and 93% at 3X by *Pseudomonas aeruginosa* PS1 (Table 29), *Enterobacter asburiae* PS2 (Table 30), *Pseudomonas putida* PS9 (Table 31) and *Klebsiella* sp. PS19, respectively (Table 32). Though the order of biotoxicity of pesticides on bacterial IAA biosynthesis was not uniform, however, at three times of recommended dose, the order of toxicity of pesticides on the IAA synthesis by *Pseudomonas aeruginosa* PS1, the most tolerant PSB strain to all herbicides, insecticides and fungicides, was quizalafop-p-ethyl > clodinafop > glyphosate > metribuzin for herbicides; pyriproxyfen > imidacloprid > fipronil > thiamethoxam for insecticides and tebuconazole > hexaconazole > metalaxyl > kitazin for fungicides (Table 29).

4.6.2 Bioassay of siderophore under pesticide stress

In the present study, production of siderophores by the pesticide tolerant strains of PGPR was also determined on CAS agar plates supplemented with or without varying concentrations of pesticides (Table 25–32). Generally, the tested PGPR strains showed siderophore activity on pesticides amended CAS agar plates. *Mesorhizobium* strain MRC4 produced a 12 mm sized colored zone on CAS plates having pesticide. The size of siderophore zone produced on CAS agar plates decreased with increasing concentrations of each pesticide. Among herbicides, both quizalafop-p-ethyl and metribuzin at normal rate reduced the zone size maximally by 9% compared to control. Clodinafop and glyphosate at normal rates, however, showed no reduction in zone size. In addition, quizalafop-p-ethyl, clodinafop and metribuzin reduced the zone size by 17% at both 2X and 3X rate but glyphosate mediated reduction was only 9% at both 2X and 3X concentrations. Fipronil, pyriproxyfen, imidacloprid and thiamethoxam (insecticides) at recommended dose, did not affect the zone size on CAS agar plates. While at 3X, fipronil, pyriproxyfen and imidacloprid at 3X equally reduced the zone size by 9%. In contrast, thiamethoxam exhibited no inhibitory effect. Among fungicides, the inhibitory effect of

tebuconazole and hexaconazole was comparatively more than those observed for metalaxyl and kitazin. Both tebuconazole and hexaconazole at 3X, decreased the zone size equally by 34% (Table 25). Furthermore, the ethyl acetate extraction from culture supernatant of *Mesorhizobium* strain MRC4 grown in the Modi medium, yielded a maximum amount of 35 and 19 µg/ml SA and DHBA in absence of pesticides. The amount of SA and DHBA, respectively, in the supernatant of mesorhizobial strains decreased consistently with increasing dose of each pesticide (Table 25). Within herbicide group, metribuzin and glyphosate poorly affected the synthesis of SA and DHBA. At 3X, both herbicides declined SA and DHBA by 15% and 6%, respectively over control. Quizalafop-p-ethyl at 3X have shown maximum toxicity and decreased SA and DHBA by 46% and 48% respectively, compared to control. Among insecticides, the most prominent inhibitory effect on SA and DHBA production was recorded for pyriproxyfen which decreased SA and DHBA by 40% and 37% at 3X. Among fungicides, tebuconazole and hexaconazole affected the siderophores production most severely. Tebuconazole reduced the production of SA and DHBA by 40% and 58% while hexaconazole by 40% and 48% respectively, at 3X over control (Table 25).

Pea specific *Rhizobium* strain MRP1 produced 11 mm colored zone on CAS agar plates under no pesticide stress. The zone size on CAS agar plates decreased with increasing concentrations of each pesticide treatment. Among herbicides, inhibitory effect of quizalafop-p-ethyl was most obvious and it decreased the zone size by 10%, 10% and 19% at X, 2X and 3X, respectively over control. In contrast, both metribuzin and glyphosate at all concentrations showed no reduction in zone size as did the four insecticides fipronil, pyriproxyfen, imidacloprid and thiamethoxam. Moreover, the 3X concentration of fipronil, pyriproxyfen, imidacloprid and thiamethoxam reduced the zone diameter by 10% compared to control while comparing the effect of fungicides, tebuconazole at X, 2X and 3X had the most inhibitory effect on siderophore activity and declined the zone size by 10, 10 and 19%, respectively (Table 26). Additionally, *Rhizobium* strain MRP1 released a highest quantity of 32 and 22 µg/ml SA and DHBA in culture supernatant devoid of pesticides. The amount of SA and DHBA in the supernatant of rhizobial strains decreased consistently with increasing dosage of each pesticide. Of all the herbicides, quizalafop-p-ethyl displayed maximum toxicity and decreased SA by 32, 41 and 57% while it reduced the DHBA by 32, 37 and 55%, respectively at X, 2X and 3X, over control. The most toxic effect on SA and DHBA production was shown, among insecticides, by pyriproxyfen

which decreased SA by 19, 29 and 35% and DHBA by 28, 37 and 46% at X, 2X and 3X, respectively. Fipronil and thiamethoxam slightly reduced the siderophore activity and showed a similar pattern of SA and DHBA inhibition following strain MRP1 inoculation. In so far as the fungicides are concerned, tebuconazole affected the siderophores production most severely and inhibited SA by 25, 38 and 44% and DHBA by 32, 55 and 60% at X, 2X and 3X, respectively, relative to control (Table 26). The qualitative and quantitative properties of siderophores were also severely affected when *Bradyrhizobium* strain MRM6 (Table 27) and *Rhizobium* strain MRL3 (Table 28) were grown in Modi medium treated differently with different concentration of herbicides, insecticides and fungicides. Quizalafop-p-ethyl among herbicides showed the substantial reduction in zone diameter by 22% (*Bradyrhizobium* strain MRM6) and 25% (*Rhizobium* strain MRL3) at three times of recommended dose. Similarly, among insecticides, pyriproxyfen at 3X showed maximum toxicity for *Bradyrhizobium* strain MRM6 and both fipronil and pyriproxyfen at 3X for *Rhizobium* strain MRL3 and reduced the siderophore zone by same degree (23%) over control. Of fungicides, tebuconazole at 3X showing most inhibitory effect on siderophore activity on CAS agar plates decreased it by 23% and 25% for *Bradyrhizobium* strain MRM6 and *Rhizobium* strain MRL3, respectively, relative to control. In addition, quizalafop-p-ethyl at 3X displayed the maximum toxicity and decreased SA by 62% and 48% and DHBA by 72% and 57% for *Bradyrhizobium* strain MRM6 and *Rhizobium* strain MRL3, respectively, over respective control. Likewise, pyriproxyfen at 3X showed maximum toxicity and decreased SA by 34% and 28% and DHBA by 33% and 57% for *Bradyrhizobium* strain MRM6 and *Rhizobium* strain MRL3, respectively, compared to their respective control. On the other hand, tebuconazole at 3X decreased both SA and DHBA by 44% and 52% for *Bradyrhizobium* strain MRM6 and *Rhizobium* strain MRL3, over their control (Table 27–28).

Phosphate solubilizing bacterial strains notably, *Pseudomonas aeruginosa* PS1, *Enterobacter asburiae* PS2, *Pseudomonas putida* PS9 and *Klebsiella* sp. PS19 tolerant to pesticides were also tested for siderophore production under pesticide stressed environment. The synthesis of siderophores by the P solubilizers both qualitatively and quantitatively decreased consistently with increasing concentrations of pesticides (Table 29–32). Generally, the zone size on CAS agar plates ranged between 11 (*Pseudomonas putida* PS9) to 15 mm (*Pseudomonas aeruginosa* PS1) in the absence of pesticides. Quizalafop-p-ethyl among herbicides displayed the maximum reduction in zone diameter by 27% (*Pseudomonas aeruginosa* PS1), 25%

(*Enterobacter asburiae* PS2), 28% (*Pseudomonas putida* PS9) and 31% (*Klebsiella* sp. PS19) at three times of recommended dose. Similarly, among insecticides, pyriproxyfen at 3X showed maximum toxicity and substantially reduced the siderophore activity by 20%, 34%, 19% and 31% as shown by strains of *Pseudomonas aeruginosa* (PS1), *Enterobacter asburiae* (PS2), *Pseudomonas putida* (PS9) and *Klebsiella* sp. (PS19), respectively. Likewise, fungicide tebuconazole at 3X exhibited most inhibitory action and decreased the zone size by 20%, 34%, 28% and 31% for *Pseudomonas aeruginosa* PS1, *Enterobacter asburiae* PS2, *Pseudomonas putida* PS9 and *Klebsiella* sp. PS19, respectively. In the absence of pesticides, maximum amount of phenolates like, SA (47 µg/ml) was produced by *Klebsiella* sp. PS19 while DHBA (21 µg/ml) was produced by *Pseudomonas aeruginosa* PS1. Generally, the released amount of bacterial SA ranged from 28 (*Enterobacter asburiae* PS2) to 47 µg/ml (*Klebsiella* sp. PS19) while DHBA varied between 9 (*Enterobacter asburiae* PS2) to 21 µg/ml (*Pseudomonas aeruginosa* PS1) in the absence of pesticide treatment. Among all herbicides, quizalafop-p-ethyl at 3X displayed the maximum decrease in production of SA by 35%, 68%, 46% and 47% and of DHBA by 48%, 78%, 89% and 90% for *Pseudomonas aeruginosa* PS1, *Enterobacter asburiae* PS2, *Pseudomonas putida* PS9 and *Klebsiella* sp. PS19, respectively, over respective control. Similarly, among insecticides, pyriproxyfen at 3X showed maximum biotoxicity to SA and decreased it by 52%, 47%, 36% and 47% and to DHBA by 80%, 83%, 67% and 70% for *Pseudomonas aeruginosa* PS1, *Enterobacter asburiae* PS2, *Pseudomonas putida* PS9 and *Klebsiella* sp. PS19, respectively, compared to their respective control. Tebuconazole (fungicide) at 3X, decreased SA to highest degree by 54%, 69%, 58% and 52% and DHBA by 70%, 77%, 67% and 77% for *Pseudomonas aeruginosa* PS1 (Table 29), *Enterobacter asburiae* PS2 (Table 30), *Pseudomonas putida* PS9 (Table 31) and *Klebsiella* sp. PS19 (Table 32), respectively over control.

4.6.3 Bioassay of exopolysaccharides under pesticide stress

Unlike other PGP substances produced by rhizobacterial strains when grown under pesticidal stress, the amount of exopolysaccharides (EPS) synthesized by all PGPR strains increased progressively with gradual enhancement in pesticide concentrations. In case of herbicides, the secretion of EPS by *Mesorhizobium* strain MRC4 in the presence of quizalafop-p-ethyl and clodinafop though did not differ significantly but metribuzin and glyphosate had the inducible effect on EPS synthesis. For instance, glyphosate at 3X, among herbicides, increased

the EPS by 23% over control. Of insecticides, imidacloprid increased EPS by 38% and fungicide hexaconazole by 33% at 3X compared to control (Table 25). For *Rhizobium* strain MRP1 specific to pea, glyphosate, pyriproxyfen and tebuconazole increased EPS by 40%, 30% and 25% respectively, at 3X over control (Table 26). On the other hand, for *Bradyrhizobium* strain MRM6, glyphosate increased EPS by 38%, fipronil, pyriproxyfen and thiamethoxam by 23%, tebuconazole and hexaconazole by 28% at 3X compared to control (Table 27). Unlike the marginal increment in EPS synthesis by *Rhizobium* strain MRL3 (lentil) in presence of metribuzin and glyphosate, both quizalafop-p-ethyl and glyphosate at 3X increased EPS by 33% when compared with untreated control. Similarly, imidacloprid at 3X increased EPS secretion in highest quantity by 44% compared to control. On the other hand, hexaconazole at three times of recommended rate, was found the most potential inducer of bacterial EPS secretion and increased it by 50% over control (Table 28). Pesticide tolerant strains of phosphate solubilizing bacteria were also tested for EPS production under pesticide stress (Table 29-32). The synthesis of EPS by the P solubilizers increased consistently with increasing concentration of each herbicides, insecticides and fungicides. The trend of EPS production by P solubilizing strains like *Pseudomonas aeruginosa* PS1, *Enterobacter asburiae* PS2, *Pseudomonas putida* PS9 and *Klebsiella* sp. PS19 under pesticide stress was not uniform. In general, the effect of glyphosate (among herbicides), pyriproxyfen (of insecticides) and hexaconazole (among fungicides) on bacterial EPS secretion was most obvious compared to their respective control. For example, glyphosate increased the synthesis of EPS by 38%, 43%, 47% and 38%, pyriproxyfen by 50%, 37%, 35% and 33% and hexaconazole by 56%, 55%, 41% and 61% at 3X by *Pseudomonas aeruginosa* PS1 (Table 29), *Enterobacter asburiae* PS2 (Table 30), *Pseudomonas putida* PS9 (Table 31) and *Klebsiella* sp. PS19 (Table 32), respectively over their respective control.

4.6.4 *In vitro* assay of ammonia and HCN under pesticide stress

The rhizobacterial strains were further tested for HCN and ammonia production under *in vitro* conditions in the presence of three concentrations (X, 2X and 3X) of twelve pesticides. Interestingly, the three concentrations of herbicides, insecticides and fungicides did not affect negatively HCN and ammonia synthesis by each *Mesorhizobium* (Table 25), *Rhizobium* specific to pea (Table 26), *Bradyrhizobium* (Table 27), *Rhizobium* specific to lentil (Table 28) and phosphate solubilizing strains of *Pseudomonas aeruginosa* PS1, *Enterobacter asburiae* PS2, *Pseudomonas putida* PS9 and *Klebsiella* sp. PS19 (Table 29–32).

4.6.5 Phosphate solubilization influenced by different group of pesticides

The phosphate solubilizing potentials of the PGPR strains in the presence of varying concentrations of herbicides, insecticides and fungicides was assayed both qualitatively and quantitatively using solid and liquid Pikovskaya medium (Table 33–36). In this study, the phosphate solubilizing bacteria, *Pseudomonas aeruginosa* (strain PS1), *Enterobacter asburiae* (strain PS2), *Pseudomonas putida* (strain PS9) and *Klebsiella* sp. (strain PS19) were used due to their inherent ability to tolerate the highest concentration of pesticides and production of PGP substances maximally. All these strains produced a largest zone of P solubilization around their growth on solid Pikovskaya medium (Table 33–36) devoid of pesticides whose solubilization index (SI) ranged between 2 (*Pseudomonas aeruginosa* PS1) and 2.5 (*Klebsiella* sp. PS19). In contrast, the zone of solubilization and *in vitro* solubilization of tri-calcium phosphate (TCP) decreased substantially when PGPR strains were grown with 3X concentrations each of herbicides, insecticides and fungicides. For example, quizalafop-p-ethyl decreased the solubilization zone by 25, 73, 58 and 45%, pyriproxyfen by 38, 50, 43 and 40% and tebuconazole by 25, 63, 58 and 60% at 3X for *Pseudomonas aeruginosa* PS1, *Enterobacter asburiae* PS2, *Pseudomonas putida* PS9 and *Klebsiella* sp. PS19 respectively, over their control. Similarly, a considerable amount of tri-calcium phosphate was solubilized in liquid culture by *Pseudomonas aeruginosa* PS1 (345 µg/ml), *Enterobacter asburiae* PS2 (258 µg/ml), *Pseudomonas putida* PS9 (298 µg/ml) and *Klebsiella* sp. PS19 (294 µg/ml) when grown in Pikovskaya medium lacking any pesticides. The amount of P solubilized however, decreased with increase in the concentration of pesticides. Similar to the effects of pesticides on solubilizing zones, a maximum reduction in P solubilization in broth was found as 96%, 82% and 96% by *Pseudomonas aeruginosa* PS1 (Table 33), 95, 87 and 94% by *Enterobacter asburiae* PS2 (Table 34), 95, 93 and 95% by *Pseudomonas putida* PS9 (Table 35) and 97, 96 and 95% by *Klebsiella* sp. PS19 (Table 36) at 3X of quizalafop-p-ethyl, pyriproxyfen and tebuconazole respectively, over their respective control.

4.7 Pesticidal Toxicity to Legumes and Rhizoremediation

4.7.1 Chickpea

4.7.1.1 Plant growth

4.7.1.1.1 Length of plant organs

The length of plant organs (roots and shoots) of chickpea grown in sandy clay loam soil treated with the recommended (X), two (2X) and three (3X) times more of recommended rates of technical grade herbicides (quizalafop-p-ethyl and clodinafop), insecticides (fipronil and pyriproxyfen) and fungicide (tebuconazole) demonstrated a variable plant growth measured at 90 and 135 days after sowing (DAS). Generally, a progressive decline with variable magnitude was observed for both root and shoot length as the concentration of all pesticides was increased from X to 3X in soil. Among herbicides, quizalafop-p-ethyl at 3X (120 µg/ kg soil) displayed the most toxic effect and significantly ($P \leq 0.05$) decreased root and shoot length by 72% (7 cm) and 53% (16 cm), respectively (at 90 DAS) (Plate 3A) and by 73% (9 cm) and 55% (17 cm), respectively (at 135 DAS) over control (Table 37). Clodinafop at 1200 µg/ kg soil (3X) decreased root length and shoot length by 42% and 21% respectively, at 90 DAS and by 40% and 28% respectively, at 135 DAS relative to control (Table 38). In contrast, the toxic effect of both insecticides (fipronil and pyriproxyfen) on root and shoot length was comparable (Table 39 and 40). The fungicide tebuconazole at 3X (300 µg/ kg soil) at 90 DAS significantly ($P \leq 0.05$) decreased root length and shoot length by 65% and 50% respectively while at 135 DAS by 64% and 49% respectively, over control (Table 41). While comparing the effects of three concentrations of each herbicide, insecticide and fungicide on the growth of plant organs, clodinafop exhibited least toxic effect while quizalafop-p-ethyl showed maximum biotoxicity to the aerial (shoots) and underground (roots) parts of chickpea plants grown in pesticide treated soils. Interestingly, when pesticide tolerant and plant growth promoting strain MRC4 of *Mesorhizobium* was also inoculated in soil amended with pesticides, the growth of roots and shoots though decreased but the effects were less pronounced. A considerable enhancement was observed in root and shoot length of inoculated chickpea plants when compared with the plants grown in soils treated solely with the similar concentration of pesticides. For example, when strain MRC4 was used with 3X of quizalafop-p-ethyl, it increased the root and shoot length by 42% and 12% respectively (at 90 DAS) and 22% and 17% respectively (at 135 DAS) compared with the uninoculated plants grown in soil treated with the same dose of quizalafop-p-ethyl (Plate 3B) (Table 37).

4.7.1.1.2 Dry biomass production

The phytotoxicity of pesticides to dry biomass production by plant organs (roots and shoots) and total dry matter accumulation in chickpea plants grown in sandy clay loam soil

consistently decreased with increasing concentrations of herbicides (Table 37, 38), insecticides (Table 39, 40) and fungicides applied separately. In general, three concentrations each of X, 2X and 3X of all pesticides significantly ($P \leq 0.05$) decreased the dry matter accumulation both at 90 DAS and 135 DAS, relative to the control. At recommended dose, quizalafop-p-ethyl (40 µg/ kg soil), clodinafop (400 µg/ kg soil), fipronil (200 µg/ kg soil), pyriproxyfen (1300 µg/ kg soil) and tebuconazole (100 µg/ kg soil) significantly ($P \leq 0.05$) reduced the root and shoot dry biomass by 41 and 71%, 10 and 10%, 38 and 41%, 24 and 55% and 48 and 63%, respectively (at 90 DAS) and by 30 and 65%, 5 and 26%, 18 and 38%, 47 and 50% and 29 and 53%, respectively (at 135 DAS) over control. Of the two herbicides, a maximum decline in plant roots (78 and 66%) and shoots (81 and 87%) dry matter was recorded at 3X (120 µg/ kg soil) of quizalafop-p-ethyl at 90 and 135 DAS, respectively (Table 37). Similarly, clodinafop at 3X (1200 µg/ kg soil) decreased plant roots (by 30 and 25%) and shoots (by 38 and 45%) biomass at 90 and 135 DAS, respectively, when compared to control (Table 38). Among insecticides, the most obvious inhibitory effect was observed for 3X (3900 µg/ kg soil) of pyriproxyfen which decreased the root dry biomass by 47% and 70% and shoot dry biomass by 73% and 66% over control at 90 and 135 DAS, respectively (Table 40). Tebuconazole (fungicide) decreased the root and shoot dry mass by 62% and 54% respectively, at 90 DAS and 78% and 72% respectively, at 135 DAS relative to control (Table 41). A similar trend for root and shoot dry mass production was observed when *Mesorhizobium* strain MRC4 was used in soil along with pesticides. However, the dry biomass of chickpea plants was significantly ($P \leq 0.05$) higher compared to those observed for uninoculated plants. Moreover, the bioinoculant (strain MRC4) significantly ($P \leq 0.05$) subsided the toxic effects of each pesticide and consequently increased the dry matter accumulation in chickpea plants at all dose rates when compared with the uninoculated plants. For instance, strain MRC4 increased the shoot dry matter by 30% (0.17 g/plant), 28% (0.29 g/plant) and 15% (0.1 g/plant) at 90 DAS and 82% (0.42 g/plant), 16% (0.27 g/plant), 14% (0.16 g/plant) at 135 DAS at three times of the recommended rates of quizalafop-p-ethyl (Table 37), fipronil (Table 39) and tebuconazole (Table 41) respectively, compared to uninoculated plants treated with the same dose of quizalafop-p-ethyl (Plate 3A, 3B), fipronil (Plate 3C, 3D) and tebuconazole (Plate 3E, 3F) respectively.

4.7.1.1.3 Symbiotic traits

4.7.1.1.3.1 Nodulation

Nodulation response to the three concentrations of quizalafop-p-ethyl, clodinafop, fipronil, pyriproxyfen and tebuconazole at 90 and 135 DAS varied considerably (Table 37, 38, 39, 40 and 41). Generally, each pesticide decreased the nodule numbers and its biomass when concentration of pesticide was increased from normal to three times more of recommended rates. Like plant dry matter accumulation, quizalafop-p-ethyl among herbicides, showed highest toxicity at three times of recommended dose (120 µg/ kg soil) and decreased nodule numbers by 67% (14/plant) and 65% (9/plant) and nodule biomass by 87% (155 mg/plant) and 79% (217 mg/plant) at 90 and 135 DAS, respectively, compared to control (Table 37). However, inhibitory effect on nodulation following X (400 µg/ kg soil), 2X (800 µg/ kg soil) and 3X (1200 µg/ kg soil) of clodinafop was non-significant in comparison to control. Clodinafop at X, 2X and 3X decreased the nodule number by 5, 10 and 24% (at 90 DAS) and 15, 9 and 36% (at 135 DAS) and nodule dry mass by 12, 19 and 27% (at 90 DAS) and 24, 36 and 55% (at 135 DAS), respectively relative to control (Table 38). Similarly, pyriproxyfen (insecticide) at 1300 µg/ kg soil (X), 2600 µg/ kg soil (2X) and 3900 µg/ kg soil (3X) adversely affected the chickpea-*Mesorhizobium* symbiosis and progressively decreased nodule numbers by 5, 10 and 14% and nodule mass by 24, 34 and 42% respectively, above the control at 90 DAS. Interestingly, pyriproxyfen at 135 DAS showed greatest toxicity and significantly ($P \leq 0.05$) decreased nodule numbers by 43, 58 and 58% and nodule dry weight by 52, 64 and 74%, at X, 2X and 3X respectively, over control (Table 40). Tebuconazole, a conazole fungicide at X (100 µg/ kg soil), 2X (200 µg/ kg soil) and 3X (300 µg/ kg soil), also severely affected nodulation and reduced nodule numbers by 24%, 34% and 48% and nodule mass by 36, 47 and 56% respectively, at 90 DAS while 3X of the same fungicide declined nodule numbers and nodule dry biomass by 36% and 73% respectively, at 135 DAS compared to control (Table 41).

Even though a trend similar to those observed for the impact of pesticides on symbiotic properties of chickpea plants was observed for inoculated plants but when strain MRC4 of *Mesorhizobium* was also applied with pesticides, it increased the nodulation (Plate 8A) efficiency of chickpea plants compared to uninoculated treatments. For example, in the presence of inoculant MRC4, fipronil at highest tested concentration (600 µg/ kg soil) decreased the nodule numbers and nodule mass by 42% and 30% respectively, at 90 DAS while at 135 DAS, it decreased nodule numbers and nodule mass by 42% and 6%, respectively (Table 39). While comparing the effect of pesticides on nodulation of chickpea plants grown both in the presence

and absence of bioinoculant, it was interesting to observe that bioinoculant, in general, significantly ($P \leq 0.05$) improved the nodulation on chickpea plants when grown even in the presence of each class of pesticides. As an example, when strain MRC4 was used with pyriproxyfen at 1300, 2600 and 3900 µg/kg soil increased the nodule numbers by 14, 31 and 16% and nodule dry mass by 25, 34 and 42% at 90 DAS while at 135 DAS, it enhanced significantly ($P \leq 0.05$) nodule numbers by 162, 233 and 183% and nodule biomass by 52, 64 and 75%, respectively (Table 40). The two-way ANOVA showed that the individual effects of inoculation and pesticides and their interaction (inoculation × pesticides) were significant ($P \leq 0.05$) for nodulation except the effect of quizalafop-p-ethyl and its interaction with inoculant MRC4 for nodule biomass at 90 DAS (Table 37); effect of clodinafop on nodule numbers at 135 DAS and nodule biomass at both 90 and 135 DAS and its interaction with inoculant at 135 DAS on nodule numbers (Table 38).

4.7.1.1.3.2 Leghaemoglobin and Chlorophyll content

The effect of three concentrations of quizalafop-p-ethyl, clodinafop, fipronil, pyriproxyfen and tebuconazole on leghaemoglobin (Lb) and chlorophyll content in nodules and fresh foliage respectively, was measured at pod fill stage (90 DAS). The leghaemoglobin and total chlorophyll content consistently declined with increasing rates of pesticides either in the presence or absence of inoculant and was significant ($P \leq 0.05$) for all pesticides (Table 42, 43, 44, 45 and 46). At highest concentration (3X) of herbicides added to soil, 120 µg/ kg soil of quizalafop-p-ethyl and 1200 µg/kg soil of clodinafop significantly ($P \leq 0.05$) decreased leghaemoglobin equally by 93% and chlorophyll by 34% and 13% respectively, above control (Table 42 and 43). In addition, pyriproxyfen (insecticide) at 3X (3900 µg/kg soil) reduced leghaemoglobin and chlorophyll content most severely by 77% and 16%, respectively, which was followed by fipronil that declined leghaemoglobin and chlorophyll content by 85% and 21%, respectively, over control (Table 44 and 45). The reduction in leghaemoglobin (93%) and chlorophyll (28%) content following 3X of tebuconazole was statistically significant ($P \leq 0.05$) over control (Table 46). Similar trend in chlorophyll and leghaemoglobin content reduction was observed when rhizobial inoculant *Mesorhizobium* strain MRC4 was used with pesticides. Nevertheless, a substantial increase in both photosynthetic pigments and leghaemoglobin was recorded when uninoculated treatments were compared with the inoculated ones used with the

same concentration of each added pesticide to soil. For instance, strain MRC4 when applied with 3X of quizalafop-p-ethyl (120 µg/ kg soil), clodinafop (1200 µg/ kg soil), fipronil (600 µg/ kg soil), pyriproxyfen (3900 µg/ kg soil) and tebuconazole (300 µg/ kg soil) increased leghaemoglobin and chlorophyll content by 0% and 45%, 130% and 26%, 133% and 32%, 150% and 32% and 100% and 37%, respectively, compared to uninoculated plants but treated with the same dose of quizalafop-p-ethyl, clodinafop, fipronil, pyriproxyfen and tebuconazole respectively.

4.7.1.1.4 Nutrient uptake and grain attributes

Nitrogen (N) and phosphorus (P) content in roots and shoots, seed yield (SY) and grain protein (GP) of chickpea plants was measured at harvest (135 DAS). The measured parameters decreased progressively with increase in the concentration of each pesticide (Table 42, 43, 44, 45 and 46). At three times of recommended dose, the percent decrease in root N, shoot N, root P, shoot P, SY and GP in presence of quizalafop-p-ethyl (at 120 µg/ kg soil) was 45, 38, 42, 39, 78 and 33; for clodinafop (at 1200 µg/ kg soil) 23, 15, 36, 29, 38 and 7; for fipronil (at 600 µg/ kg soil) 28, 15, 30, 24, 52 and 9; for pyriproxyfen (at 3900 µg/ kg soil) 28, 26, 42, 29, 60 and 18 and for tebuconazole (at 300 µg/ kg soil) 34, 38, 30, 34, 67 and 24, respectively, compared to the control. In addition, when *Mesorhizobium* strain MRC4 was used with pesticides, similar pattern of toxicity on nutrient and yield parameters was detected and order of toxicity was: quizalafop-p-ethyl > tebuconazole > pyriproxyfen > fipronil > clodinafop. In contrast, the inoculated strain significantly ($P \leq 0.05$) increased the root N, shoot N, root P, shoot P, SY and GP at all concentration of pesticides. For example, the rhizobial inoculant (strain MRC4) when used with 3X of fipronil, increased the root N, shoot N, root P, shoot P, SY and GP by 46, 21, 58, 37, 123 and 11%, respectively, when compared with the plants grown in soils solely treated with insecticide. The two-way ANOVA in general, showed that the individual effects of inoculation and pesticides and their interaction (inoculation × pesticides) were significant ($P \leq 0.05$) for all the measured parameters.

4.7.2 Pea

4.7.2.1 Plant growth

4.7.2.1.1 Length of plant organs

Pea plants grown in sandy clay loam soil treated with three concentrations each of quizalafop-p-ethyl, clodinafop (Plate 4A, 4B), fipronil, pyriproxyfen (Plate 4C, 4D) and tebuconazole 90 and 120 DAS showed variable plant growth. A pattern of progressive decline with variable degree was noted for both root and shoot length as the concentrations of three classes of pesticides were increased in soils. Among herbicides, quizalafop-p-ethyl at X, 2X and 3X demonstrated highest toxicity and significantly ($P \leq 0.05$) decreased root length by 35, 45 and 55% respectively, at 90 DAS and by 63, 75 and 78% respectively, at 120 DAS while shoot length by 30, 38 and 50% respectively (at 90 DAS) and 45, 62 and 62% respectively (at 120 DAS) over control (Table 47). Conversely, the effect of clodinafop was least pronounced on root and shoot length. For instance, clodinafop at 3X concentration, decreased the root length by 20% and 41% while shoot length by 30% and 31% respectively at 90 DAS (Plate 4A) and 120 DAS respectively, relative to control (Table 48). Of insecticides, effect of pyriproxyfen was more deleterious in comparison to fipronil. Pyriproxyfen at thee times more of recommended rate, decreased root and shoot length by 35% and 17% respectively, at 90 DAS and by 71% and 62% respectively, at 120 DAS compared to control (Table 49). Tebuconazole at 3X also adversely affected the growth of plant organs (roots and shoots) (Plate 4E, 4F) and it decreased root and shoot length by 35% and 30% respectively, at 90 DAS and by 75% and 64% respectively, 120 DAS over control (Table 51). The decreasing order of phytotoxicity of pesticides on the length of plant organs was: quizalafop-p-ethyl > tebuconazole > pyriproxyfen > fipronil > clodinafop. A substantial improvement was observed in root and shoot length of pea plants inoculated with *Rhizobium* strain MRP1 when compared with the uninoculated but treated with the same concentration of pesticides. For example, strain MRP1 when used with three times more of recommended dose of tebuconazole increased the root and shoot length by 38% and 5% respectively, at 90 DAS and 43% and 30% respectively, at 120 DAS, when compared with the uninoculated plants and treated with the same dose of tebuconazole (Table 51).

4.7.2.1.2 Dry biomass production

All pesticides adversely affected the dry matter accumulation in roots and shoots as well as total dry biomass of pea plants grown in pesticide amended soil. The dry biomass of roots and shoots continuously decreased as the concentration of each pesticide was increased from recommended to three times more of recommended dose (Table 47, 48, 49, 50 and 51). Usually, each concentration significantly ($P \leq 0.05$) decreased the dry mass accumulation both at 90 DAS

and 120 DAS, over the control. For example, among herbicides, quizalafop-p-ethyl at recommended dose significantly ($P \leq 0.05$) decreased roots and shoots dry mass by 49% (0.17 g/plant) and 51% (0.70 g/plant) respectively (Table 47), while clodinafop decreased root and shoot dry mass by 9% (0.03 g/plant) and 8% (0.10 g/plant) respectively (Table 48), at 90 DAS compared to control. Among insecticides, pyriproxyfen decreased roots and shoots dry mass by 29% and 37% respectively (Table 50), while fipronil mediated decline in roots and shoots dry biomass was 18% and 34% respectively (Table 49), at normal rate at 90 DAS over control. Moreover, reduction in roots and shoots dry biomass by fungicide tebuconazole at X was 38% and 54% respectively at 90 DAS, relative to control (Table 51). Like the toxicity of pesticides on dry biomass of roots and shoots at 90 DAS, a similar trend was also observed at 120 DAS. Additionally, the decreasing order of toxicity of pesticides on root and shoot biomass accumulation was: quizalafop-p-ethyl > tebuconazole > pyriproxyfen > fipronil > clodinafop. When bioinoculant *Rhizobium* strain MRP1 was also used with recommended dose, a decrease in roots and shoots dry biomass was 33% each (for quizalafop-p-ethyl), 11% and 8% (for clodinafop), 15% and 15% (for fipronil), 15% and 19% (for pyriproxyfen) and 18% and 23% (for tebuconazole) respectively, at 90 DAS while at 120 DAS it was 35% and 20% (for quizalafop-p-ethyl), 7% and 13% (for clodinafop), 7% and 13% (for fipronil), 16% and 30% (for pyriproxyfen) and 16% and 8% (for tebuconazole) respectively, compared to control (Table 47, 48, 49, 50 and 51). In general, greatest decline in plant roots and shoots dry biomass was shown by quizalafop-p-ethyl and this was followed by tebuconazole at both 90 and 120 DAS (Table 47 and 51). On the contrary, clodinafop mediated decline in dry mass accumulation was least pronounced compared to other pesticides (Table 48). The trend of reduction in dry biomass of roots and shoots under pesticide stress was similar for both inoculated and uninoculated plants. However, in the presence of bioinoculant, the severity of pesticide generated toxicity on biomass accumulation was substantially decreased. For instance, strain MRP1 when used with 3X of tebuconazole, significantly increased the root dry matter by 81% and shoot dry mass by 60% at 90 DAS while at 120 DAS, it considerably increased root dry mass by 40% and shoot dry weight by 56%, compared to uninoculated plants but treated with the same dose of tebuconazole (Table 51).

4.7.2.2 Symbiotic traits

4.7.2.2.1 Nodulation

A substantial variation was observed in nodule numbers and nodule dry mass of pea plants raised in soils amended with three concentrations each of quizalafop-p-ethyl, clodinafop, fipronil, pyriproxyfen and tebuconazole at both 90 and 120 DAS (Table 47, 48, 49, 50 and 51). Generally, with increase in the concentration of pesticides, there was a decrease in nodulation on pea plants. In case of some pesticides (e.g. quizalafop-p-ethyl), nodule formation was so much adversely affected that not a single nodule was recovered from roots of pea plants treated with higher concentration of pesticides. Among herbicides, quizalafop-p-ethyl showed maximum toxicity and significantly ($P \leq 0.05$) decreased nodule numbers and nodule dry weight by 75% and 48% respectively, at 90 DAS over the control while at 120 DAS it completely diminished nodulation (Table 47). In contrast, clodinafop at X marginally decreased nodule numbers and nodule biomass both at 90 and 120 DAS (Table 48). Of the insecticides, pyriproxyfen at X exhibited a significant toxic effect on nodulation and decreased nodule numbers and their biomass by 41% and 25% respectively, at 90 DAS whereas at 120 DAS by 40% and 62% respectively, compared to control (Table 50). On the other hand, fipronil at X decreased numbers and dry mass of nodules by 45% and 17% respectively, at 90 DAS while at 120 DAS by 40% and 47% respectively, compared to control (Table 49). Moreover, percent decrease in nodule number and their dry weight in the presence of recommended rate of tebuconazole was 30 and 11 respectively, at 90 DAS and 40 and 25 respectively, at 120 DAS in comparison to control (Table 51). Furthermore, all pesticides at 3X completely suppressed the nodule formation at 120 DAS. In general, quizalafop-p-ethyl, among all pesticides displayed the most lethal effect on nodule formation (Table 47). Moreover, bioinoculant MRP1 significantly ($P \leq .05$) improved the nodulation (Plate 8B) on pea plants when grown even in the presence of pesticides. For instance, strain MRP1 applied with 200 µg tebuconazole /kg of soil increased nodule numbers by 11% and nodule dry mass by 47% at 90 DAS compared to uninoculated but treated with the same dose of tebuconazole (Table 51). Moreover, the two-factor ANOVA revealed that the individual effects of inoculation and pesticides and their interactive effect (inoculation × pesticides) were significant ($P \leq 0.05$) for nodulation except the effect of quizalafop-p-ethyl and its interaction with inoculant MRP1 for nodule biomass at 90 DAS (Table 47); effect of clodinafop on nodule numbers at 120 DAS and nodule biomass at 90 DAS and its interaction with inoculant on nodule numbers at 120 DAS and nodule biomass both at 90 and 120 DAS (Table 48); interactive effect

of both fipronil (Table 49) and pyriproxyfen (Table 50) on nodule dry biomass at 90 DAS and interactive effect of tebuconazole on nodule numbers at 90 DAS.

4.7.2.2.2 Leghaemoglobin and chlorophyll content

Leghaemoglobin content in fresh nodules and total chlorophyll content in foliage measured at 90 DAS, progressively decreased with increasing concentration of each pesticide both in the presence or the absence of bioinoculant. Quizalafop-p-ethyl, clodinafop, fipronil, pyriproxyfen and tebuconazole at 3X decreased leghaemoglobin and chlorophyll content by 100 and16%, 24 and 8%, 36 and 10%, 36 and 15% and 100 and 14% respectively, over control (Table 52, 53, 54, 55 and 56). Similarly, leghaemoglobin and chlorophyll contents were also reduced in a similar manner when inoculant *Rhizobium* strain MRP1 was inoculated with pesticides. However, a substantial increase in leghaemoglobin and chlorophyll content was observed when inoculated treatments were compared with the uninoculated ones following the same concentration of pesticides. For illustration, strain MRP1, when used with fipronil (at three times more of recommended dose), increased leghaemoglobin and chlorophyll content by 36% and 19% respectively, compared to uninoculated plants [leghaemoglobin 0.23 mM (g f. m.)$^{-1}$; chlorophyll 0.89 mg/g] treated with the same dose of fipronil (Table 54).

4.7.2.3 Nutrient uptake and grain attributes

Nitrogen and P content, SY and GP of pea plants measured at harvesting, decreased gradually with increasing concentrations of each pesticide (Table 52, 53, 54, 55 and 56). At three times of recommended dose, the percent decrease in root N, shoot N, root P, shoot P, SY and GP was 24, 36, 39, 36, 50 and 4 for quizalafop-p-ethyl (Table 52); 15, 29, 24, 18, 14 and 2 for clodinafop (Table 53); 27, 27, 29, 18, 15 and 2 for fipronil (Table 54); 27, 20, 29, 25, 15 and 2 for pyriproxyfen (Table 55) and 26, 32, 34, 33, 23 and 3 for tebuconazole (Table 56), respectively, compared to the control. In addition, when strain MRP1 was used with pesticides, similar trend was observed for the same parameters but severity of toxicity of all pesticides on these parameters was less pronounced. Moreover, quizalafop-p-ethyl was found as most toxic followed by tebuconazole. In contrast, the inoculant strain significantly ($P \leq 0.05$) increased the root N, shoot N, root P, shoot P, SY and SP at all concentration of pesticides. For instance, the inoculant when used with tebuconazole at 3X, increased the root N, shoot N, root P, shoot P, SY and SP by 20, 19, 50, 31, 16 and 7%, respectively, compared to the uninoculated plants treated with the same dose of tebuconazole (Table 56). Generally, the two-way ANOVA revealed that

the individual effects of inoculation and pesticides and their interaction (inoculation × pesticides) were significant ($P \leq 0.05$) for all the measured parameters.

4.7.3 Greengram

4.7.3.1 Plant growth

4.7.3.1.1 Root length and shoot length

Uninoculated and *Bradyrhizobium* inoculated greengram plants grown in soil treated separately with three concentrations each of quizalafop-p-ethyl (Plate 5A, 5B), clodinafop, fipronil, pyriproxyfen and tebuconazole (Plate 5D, 5E) showed a considerable variation in pot house experiments. The length of roots and shoots of greengram plants declined consistently following increase in the concentration of pesticides (Table 57, 58, 59, 60 and 61). However, no significant differences were observed on the measured parameters while comparing the effect of pesticides on 50 DAS or 80 DAS old greengram plants. For instance, clodinafop at 3X, decreased the root length by 65% at 50 DAS (Plate 6A) while at 80 DAS, it decreased root length by 64%. Similarly, 3X of clodinafop decreased shoot length by 50 and 63% at 50 and 80 DAS, respectively relative to control (Table 58). On the contrary, fipronil at 3X decreased root length by 50% each at 50 (Plate 6B) and 80 DAS while shoot length declined by 36% and 50% at 50 and 80 DAS, respectively over control (Table 59). For plant growth promoting and pesticide tolerant *Bradyrhizobium* (strain MRM6) inoculated plants, though the root and shoot length decreased continuously with increasing concentration of pesticides but a significant ($P \leq 0.05$) enhancement was found in the measured parameters of greengram plants when compared with the uninoculated but treated with the same concentration of pesticides. For example, when strain MRM6 was used with 3X of tebuconazole, it increased both root (by 36% and 37% at 50 and 80 DAS, respectively) and shoot (36% and 38% at 50 and 80 DAS, respectively) length when inoculated greengram plants grown in soils treated with 3X of tebuconazole were compared with the uninoculated plants grown in soils treated with the same rate of tebuconazole (Table 61).

4.7.3.1.2 Dry biomass production

The dry matter accumulation in roots, shoots and whole greengram plants were adversely affected in response to pesticidal exposure. The plant organs (roots and shoots) biomass continuously decreased with increase in the concentration of pesticides (Table 57, 58, 59, 60 and

61). Generally, all concentrations of pesticides significantly ($P \leq 0.05$) decreased the dry matter accumulation of whole greengram plants both at 50 and 80 DAS, relative to control. Generally, the dry matter accumulation in shoots of greengram plants was comparatively higher than those observed for roots. Quizalafop-p-ethyl (Table 57), clodinafop (Table 58), fipronil (Table 59), pyriproxyfen (Table 60) and tebuconazole (Table 61) at recommended rate significantly ($P \leq 0.05$) decreased roots dry mass by 60, 47, 52, 71 and 16% respectively, while shoots dry mass decreased by 51, 48, 36, 40 and 26% respectively, at 50 DAS. Similarly, the same pesticides at the same rate decreased the roots dry mass by 62, 50, 36, 46 and 21% respectively, while shoots dry mass by 43, 7, 36, 37 and 36% respectively, at 80 DAS compared to control (roots dry biomass 0.47 g/plant and shoots dry biomass 2.08 g/plant). The pesticidal toxicity onto greengram plants increased in the order: quizalafop-p-ethyl > tebuconazole > pyriproxyfen > fipronil > clodinafop. When pesticide tolerant *Bradyrhizobium* strain MRM6 was also inoculated in pesticide amended soil, quizalafop-p-ethyl, clodinafop, fipronil, pyriproxyfen and tebuconazole at all concentrations, decreased root and shoot dry matter like uninoculated treatments but the toxicity of pesticides to plant biomass was greatly mitigated compared to uninoculated treatments. Consequently, roots and shoots dry mass at each dose rate of all pesticides increased appreciably when inoculated plants were compared with the uninoculated plants but treated with the same dose rate of each pesticide. For example, strain MRM6 when applied with 1200 µg/kg of clodinafop, significantly ($P \leq 0.05$) increased the root and shoot dry matters by 68% and 61% respectively, at 50 DAS and 62% and 24% at 80 DAS respectively, compared to uninoculated greengram plants grown in soils treated with the same concentration of clodinafop (Table 58).

4.7.3.2 Symbiotic traits

4.7.3.2.1 Nodulation

When greengram plants were grown in soils treated with quizalafop-p-ethyl, clodinafop, fipronil, pyriproxyfen and tebuconazole, a substantial variation in nodulation was observed for both 50 and 80 old grown plants (Table 57, 58, 59, 60 and 61). Generally, with increasing concentrations of pesticide, the symbiotic traits of greengram plants decreased progressively in both the presence and absence of bioinoculant. For uninoculated plants, quizalafop-p-ethyl (Table 57), clodinafop (Table 58), fipronil (Table 59), pyriproxyfen (Table 60) and tebuconazole (Table 61) at recommended dose caused a substantial abatement in nodule numbers by 29, 10,

10, 15 and 17%, while nodule dry weight by 38, 8, 13, 24 and 25% respectively, at 50 DAS relative to control. Similarly, at 80 DAS the same pesticides at recommended rate decreased the nodule numbers by 73, 6, 6, 12 and 24% and nodule dry mass by 61, 10, 10, 18 and 16% respectively, over control. Among all the pesticide, quizalafop-p-ethyl in general, displayed the most lethal effect on nodulation (Table 57). Moreover, bioinoculant strain MRM6 increased the nodule numbers and their mass extensively at all concentrations of each pesticide. For instance, MRM6 with 3X of pyriproxyfen increased nodule numbers and nodule dry mass by 33% and 172% respectively, at 50 DAS while at 80 DAS by 62% and 153% respectively, when compared to the uninoculated plants grown in soils treated with 3X of pyriproxyfen (Table 60). Furthermore, the two-way ANOVA revealed that the individual effects of inoculation and pesticides and their interaction (inoculation × pesticides) were significant ($P \leq 0.05$) for nodulation excluding the effect of inoculant MRM6, clodinafop and their interaction on nodule numbers at 80 DAS (Table 58); effect of tebuconazole and inoculation at 80 DAS and their interaction both at 50 and 80 DAS on nodule numbers (Table 61).

4.7.3.2.2 Leghaemoglobin and chlorophyll content

Leghaemoglobin and chlorophyll content measured at 50 DAS, declined consistently with increasing concentration of each pesticide both in the presence and absence of rhizobial inoculant (Table 62, 63, 64, 65 and 66). For instance, quizalafop-p-ethyl (Table 62), clodinafop (Table 63), fipronil (Table 64), pyriproxyfen (Table 65) and tebuconazole (Table 66) at 3X decreased leghaemoglobin and chlorophyll content by 63 and 25%, 38 and 9%, 38 and 13%, 50 and 14% and 50 and 15% respectively, relative to control. For *Bradyrhizobium* strain MRM6 inoculated plants, leghaemoglobin and chlorophyll content were also reduced in a similar way as observed for uninoculated plants. Nevertheless, a considerable increase in leghaemoglobin and chlorophyll content was observed when inoculated plants were compared with the uninoculated ones but treated with same concentration of pesticide. For example, when *Bradyrhizobium* strain MRM6 was applied with clodinafop at two times more of recommended dose, increased leghaemoglobin and chlorophyll content by 33% and 14% respectively, compared to uninoculated plant but grown with the same dose of clodinafop (Table 63).

4.7.3.3 Nutrient uptake and grain attributes

Nitrogen (N) and phosphorus (P) content, seed yield (SY) and grain protein (GP) measured at 80 DAS decreased regularly with increasing the dose rate of each pesticide (Table

62, 63, 64, 65 and 66) both in the presence and the absence of inoculant. At three times of recommended rate, the percent decrease in root N, shoot N, root P, shoot P, SY and GP in the presence of quizalafop-p-ethyl was 45, 44, 52, 37, 63 and 12 (Table 62); 17, 16, 15, 20, 29 and 4 for clodinafop (Table 63); 34, 22, 23, 14, 38 and 5 for fipronil (Table 64); 37, 32, 38, 25, 40 and 7 for pyriproxyfen (Table 65) and 25, 30, 38, 34, 49 and 8 for tebuconazole (Table 66), respectively, compared to control. In addition, when strain MRM6 was also applied as inoculant in pesticide amended soil, a similar pattern of decline in N and P content, SY and GP was observed but the toxicity of each pesticide was less pronounced in comparison to uninoculated plants. Generally, quizalafop-p-ethyl was most toxic followed by tebuconazole in decreasing nutrient and yield parameters. Interestingly, the inoculant strain significantly ($P \leq 0.05$) increased the root N, shoot N, root P, shoot P, SY and GP at all concentration of pesticides. For instance, the inoculant (MRM6) when used with 3X of fipronil, significantly ($P \leq 0.05$) increased the root N, shoot N and SY by 29, 31 and 78% respectively, compared to the plants raised with 3X of fipronil but without inoculant (Table 64). Commonly, the two-way ANOVA showed that the individual effects of inoculation and herbicides/insecticides/fungicides and their interaction (inoculation × pesticides) were significant ($P \leq 0.05$) for the measured parameters.

4.7.4 Lentil

4.7.4.1 Plant growth

4.7.4.1.1 Roots length and shoots length

The effect of three concentrations of herbicides [quizalafop-p-ethyl (Plate 7A, 7F), clodinafop (Plate 7B)], insecticides [fipronil (Plate 7C) and pyriproxyfen (Plate 7D)] and fungicide [tebuconazole (Plate 7E)] on the growth of roots and shoots of lentil plants was assessed at 90 and 120 DAS. All pesticides in general showed the phytotoxicity and reduced the length of both roots and shoots progressively with increase in the concentration of the pesticides. A dose-dependent decrease in root and shoot length following pesticidal exposure to lentil plants did not change even when pesticide tolerant and plant growth promoting *Rhizobium* strain MRL3 was used as bioinoculant. However, the decline in measured parameters was less pronounced in comparison to uninoculated but pesticide treated lentil plants (Table 67, 68, 69, 70 and 71). In general, quizalafop-p-ethyl affected most negatively the growth of roots and shoots of lentil plants. Moreover, the effects of other pesticides like fipronil, pyriproxyfen and tebuconazole on

root and shoot length was comparable. In contrast, clodinafop brought about least changes in plant growth attributes relative to other pesticides at recommended dose but at higher concentrations, effect of clodinafop was closely related to other pesticides except quizalafop-p-ethyl. For example, quizalafop-p-ethyl at 3X had a profound toxic effect and significantly ($P \leq 0.05$) decreased root length and shoot length by 83 and 20% respectively, at 90 DAS while at 120 DAS by 48 and 68% respectively, over control (Table 67). Similarly, clodinafop at 3X profoundly decreased root length and shoot length by 48 and 25% respectively, at 90 DAS while at 120 DAS by 24 and 29% respectively compared to control (Table 68). However, when *Rhizobium* strain MRL3 was employed with pesticides, decline in root and shoot length was less obvious compared to uninoculated plants. A substantial increase in root and shoot length of inoculated lentil plants occurred compared to the uninoculated plants but grown in the soils treated with the same concentration of pesticides. For example, strain MRL3 in the presence of tebuconazole (at three times of recommended dose), increased the root and shoot length by 43 and 27% respectively, at 90 DAS while at 120 DAS by 8 and 17% respectively, compared to the uninoculated plants grown in the soil treated with the same dose of tebuconazole (Table 71).

4.7.4.1.2 Dry biomass production

In general, dry biomass production by roots and shoots and total dry matter accumulation in lentil plants progressively decreased with increasing dose of each pesticide at both 90 and 120 DAS, compared to control (Table 67, 68, 69, 70 and 71). At recommended dose, quizalafop-p-ethyl (Table 67), clodinafop (Table 68), fipronil (Table 69), pyriproxyfen (Table 70) and tebuconazole (Table 71) decreased roots and shoots dry biomass by 40 and 45%, 10 and 8%, 19 and 19%, 32 and 24% and 28 and 36% respectively, at 90 DAS while at 120 DAS by 52 and 41%, 7 and 6%, 22 and 11%, 26 and 16% and 32 and 24% respectively, over control. A maximum decline in plant roots (68 and 71%) and shoots (65 and 63%) dry matter was shown by quizalafop-p-ethyl (3X) at 90 and 120 DAS, respectively (Table 55). The toxicity of other pesticides to lentil plants was however, comparatively less visible. For example, fungicidal tebuconazole at 3X significantly ($P \leq 0.05$) decreased roots and shoots dry mass by 66 and 52% respectively, at 90 DAS while at 120 DAS by 66 and 51% respectively, relative to control (Table 71). In contrast, clodinafop gave a least toxic effect compared to other pesticides (Table 68). Similar trend in roots and shoots dry biomass production was observed when *Rhizobium* strain MRL3 was used with pesticides but dry mass content of inoculated plants was much higher in

comparison to those observed for uninoculated plants. For instance, 2X each of fipronil and pyriproxyfen in the presence of bioinoculant significantly ($P \leq .05$) decreased the dry matter accumulation in roots by 40 and 50% respectively, at 90 DAS while at 120 DAS by 11 and 19% respectively. Similarly, 2X each of fipronil and pyriproxyfen decreased shoot dry mass by 30 and 43% respectively, at 90 DAS while at 120 DAS by 20 and 17% respectively, relative to control (Table 69 and 70). Furthermore, strain MRL3 significantly ($P \leq .05$) increased the dry biomass at all dose rates of pesticides when inoculated lentil plants were compared with the uninoculated plants. For example, strain MRL3 along with X, 2X and 3X of clodinafop increased the shoot dry matters by 67, 80 and 60% respectively, at 90 DAS while at 120 DAS by 76, 75 and 80%, compared to uninoculated plants grown in soils treated with the same dose rates of clodinafop (Table 68).

4.7.4.2 Symbiotic traits

4.7.4.2.1 Nodulation

Nodulation in lentil plants grown in soils treated with the three concentrations each of quizalafop-p-ethyl, clodinafop, fipronil, pyriproxyfen and tebuconazole at 90 and 120 DAS differed considerably (Table 67, 68, 69, 70 and 71). Each pesticide depressed progressively the nodule numbers and their dry weight following increase in concentration of pesticides. Among the pesticides, clodinafop at 3X displayed least toxic effect on nodulation and decreased the nodule numbers by 37% (at 90 DAS) and by 27% (at 120 DAS) and nodule dry mass by 30% (at 90 DAS) and 32% (at 120 DAS) (Table 68). On the contrary, quizalafop-p-ethyl at 3X showed maximum toxicity among pesticides at both 90 and 120 DAS (Table 67). Similarly, tebuconazole at 3X significantly ($P \leq .05$) depressed lentil-*Rhizobium* symbiosis and decreased both nodule numbers and nodule mass by 100% above the control at 90 DAS (Table 71). Similar trend for nodule numbers and nodule dry mass of lentil plants was observed when *Rhizobium* strain MRL3 as bioinoculant was also used with pesticides. For example, in the presence of inoculant MRL3, pyriproxyfen at 3X decreased the nodule numbers by 33% and nodule dry mass by 63% at 90 DAS while at 120 DAS, decreased nodule numbers by 53% and nodule dry matter by 41%, over control (Table 70). However, the bioinoculant significantly ($P \leq .05$) increased the nodule numbers and nodule biomass when compared to uninoculated plants grown with the same concentration of pesticide. For example, strain MRL3 with fipronil at X, 2X and 3X, increased nodule numbers by 50%, 38% and 50% respectively, and nodule dry mass by 108%, 86% and

84% respectively, at 90 DAS. At 120 DAS, nodule numbers did not increase significantly ($P \leq .05$) but nodule biomass was enhanced significantly by 23, 36 and 60%, respectively, compared to the uninoculated plants treated with the same dose of fipronil (Table 69). Also statistically, it was concluded employing the two-way ANOVA that the individual effects of inoculation and pesticides and their interaction (inoculation × pesticides) were significant ($P \leq 0.05$) for nodulation except the interactive effect of inoculant MRL3 with pyriproxyfen on nodule numbers at 120 DAS (Table 70).

4.7.4.2.2 Leghaemoglobin and chlorophyll content

Quizalafop-p-ethyl, clodinafop, fipronil, pyriproxyfen and tebuconazole also adversely affected leghaemoglobin and chlorophyll content measured at 90 DAS. The leghaemoglobin and total chlorophyll content consistently decreased with increasing concentrations of pesticides (Table 72, 73, 74, 75 and 76). At recommended dose, quizalafop-p-ethyl (Table 72), fipronil (Table 74), pyriproxyfen (Table 75) and tebuconazole (Table 76) decreased leghaemoglobin and chlorophyll content by 100 and 22%, 17 and 7%, 25 and 4% and 34 and 13% respectively, relative to control. The same decreasing trend in leghaemoglobin and chlorophyll content in the presence of pesticides was also observed when inoculant *Rhizobium* strain MRL3 was used with pesticides. However, substantial increase in leghaemoglobin and chlorophyll content was observed when inoculated plants were compared with the uninoculated ones amended with same concentration of pesticides. For example, strain MRL3 with clodinafop (at three times of recommended dose) increased leghaemoglobin and chlorophyll content by 50% and 14%, respectively compared to uninoculated plants treated with the same dose of clodinafop (Table 73).

4.7.4.3 Nutrient uptake and grain attributes

Nitrogen (N) and phosphorus (P) content, seed yield (SY) and grain protein (GP) of lentil plants measured at harvest (120 DAS) decreased progressively with increasing concentration of each pesticide (Table 72, 73, 74, 75 and 76). At recommended dose, the percent decrease in root N, shoot N, root P, shoot P and SY in the presence of quizalafop-p-ethyl was 30, 16, 24, 25 and 57 (Table 72); 6, 5, 5, 8 and 14 for clodinafop (Table 73); 12, 7, 15, 15 and 27 for fipronil (Table 74); 18, 9, 20, 22 and 40 for pyriproxyfen (Table 75) and 24, 14, 20, 15 and 60 for tebuconazole (Table 76), respectively, compared to control. Additionally, when strain MRL3 was also inoculated with pesticides, similar trend of dose dependent inhibition in N and P

content, SY and SP was observed but severity of pesticide toxicity to these parameters was less pronounced. In general, quizalafop-p-ethyl was comparatively more toxic than other pesticides (Table 72). However, the inoculanted strain significantly ($P \leq 0.05$) increased the root N, shoot N, root P, shoot P, SY and SP at all concentration of pesticides compared to uninoculated plants. For example, the rhizobial inoculant when used with pesticides, significantly ($P \leq .05$) increased the root N, root P, shoot P and SY by 30, 41, 21 and 55%, respectively, at 3X of clodinafop (Table 73) and 33, 61, 26 and 111%, respectively, at 3X of tebuconazole (Table 76) when compared to the treatments with the same dose of pesticides but devoid of inoculant. The two-factor ANOVA of the measured parameters of lentil plants revealed that the individual effects of inoculation and pesticides and their interaction (inoculation × pesticides) in general, were significant ($P \leq 0.05$).

4.7.5 Effect of phosphate solubilizing *P. aeruginosa* on biological and chemical properties of greengram

4.7.5.1 Plant growth

4.7.5.1.1 Root length and shoot length

Extent of toxicity generated by herbicides (quizalafop-p-ethyl and clodinafop), insecticides (fipronil and pyriproxyfen) and fungicides (tebuconazole) on the growth and productivity of greengram was assessed both at 50 and 80 DAS. Whether uninoculated or inoculated, pesticides treated plants showed significant difference in plant growth. Roots and shoots length of greengram plants progressively decreased on increasing the concentration of pesticides from recommended to three times more of recommended rate (Table 77, 78, 79, 80 and 81). In general, most toxic effect on plant growth was shown by 3X of quizalafop-p-ethyl (Plate 5A) that significantly ($P \leq .05$) decreased the roots and shoots length by 68% and 65% respectively, at 50 DAS while at 80 DAS by 67% and 67% respectively, over control (Table 77). Tebuconazole, among pesticides, displayed minimum toxicity on the same plant organs (Plate 5D) on increasing rate of pesticides and it decreased the root and shoot length equally by 36% at 50 DAS and at 80 DAS by 37% and 38% respectively, relative to control (Table 81). Inhibitory effect of pesticides on roots and shoots growth was considerably reduced when pesticide tolerant *Pseudomonas aeruginosa* strain PS1 was inoculated with seeds following pesticides application (Plate 5C, 5F). Interestingly, significant enhancement in length of both roots and shoots was observed when pesticides treated inoculated plants were compared with the uninoculated ones

treated with the same concentration of pesticides. For illustration, the increase in root and shoot length following *Pseudomonas aeruginosa* strain PS1 with fipronil (at 3X) was statistically significant ($P \leq .05$) and the inoculant with 3X of the same insecticide enhanced the root and shoot length by 100% and 123% respectively, at 50 DAS while at 80 DAS by 80% and 250% respectively, compared to uninoculated plants but treated with the same dose of fipronil (Table 79).

4.7.5.1.2 Dry biomass production

Also, dry biomass of plant organs (roots and shoots) decreased significantly ($P \leq .05$) both at 50 DAS and 80 DAS, when greengram plants were treated with the increasing rate of each pesticide (Table 77, 78, 79, 80 and 81). Quizalafop-p-ethyl most adversely affected the dry biomass production of roots and shoots of greengram plants. For instance, quizalafop-p-ethyl at recommended dose, decreased roots and shoots dry biomass by 60% and 62% respectively, at 50 DAS while at 80 DAS, by 62% and 43% respectively, over control (Table 77). The toxic effect of pesticides on dry biomass of roots and shoots of the inoculated plants was greatly reduced compared to uninoculated treatments. Moreover, at all dose rate of pesticides, roots and shoots dry biomass increased substantially when inoculated plants were compared with the uninoculated plants. For example, bioinoculant *Pseudomonas aeruginosa* strain PS1 with 3X of pyriproxyfen increased the roots dry matter by 247% and shoots dry weight by 413% at 50 DAS while at 80 DAS, it dramatically increased roots dry biomass by 447% and shoot dry weight by 513% compared to uninoculated greengram plants treated with the same concentration of pyriproxyfen (Table 80).

4.7.5.2 Symbiotic traits

4.7.5.2.1 Nodulation

A substantial decrease in numbers of nodules and their dry biomass was observed at 50 and 80 DAS when greengram plants were grown in soils treated with increasing concentrations of quizalafop-p-ethyl, clodinafop, fipronil, pyriproxyfen and tebuconazole (Table 77, 78, 79, 80 and 81). Quizalafop-p-ethyl (Table 77), clodinafop (Table 78), fipronil (Table 79), pyriproxyfen (Table 80) and tebuconazole (Table 81) at recommended dose decreased nodule numbers by 29, 10, 10, 15 and 24%, respectively and nodule dry mass by 38, 8, 13, 19 and 25% respectively, at 50 DAS while at 80 DAS, nodule numbers by 24, 6, 6, 12, 24% and nodule dry mass by 41, 10, 10, 18 and 16% respectively, over the control. In general, quizalafop-p-ethyl mediated

toxicity on nodulation of greengram plants was most pronounced in comparison to other pesticides (Table 77). In addition, bioinoculant strain PS1 increased the nodule numbers and their mass significantly in the presence of all three concentrations of each pesticide (Plate 9A, 9B). For illustration, *Pseudomonas aeruginosa* strain PS1with clodinafop at 3X increased nodule numbers significantly ($P \leq 0.05$) by 156% and nodule dry mass by 178% at 50 DAS while at 80 DAS, nodule numbers by 63% and nodule dry mass by 293% compared to the uninoculated greengram plants treated with the same dose of clodinafop (Table 78). Moreover, the two-way ANOVA showed that the individual effects of inoculation and pesticides and their interaction (inoculation × pesticides) were significant ($P \leq 0.05$) for nodulation.

4.7.5.2.2 Leghaemoglobin and chlorophyll content

Leghaemoglobin and chlorophyll content progressively decreased with increasing concentrations of each pesticide both in the presence or the absence of the inoculant. Quizalafop-p-ethyl (Table 82), clodinafop (Table 83), fipronil (Table 84), pyriproxyfen (Table 85) and tebuconazole (Table 86) at three times more of recommended rate decreased leghaemoglobin and chlorophyll content by 63 and 25%, 38 and 9%, 38 and 13%, 50 and 14% and 50 and 15% respectively, over control. In the presence of the inoculant strain PS1, leghaemoglobin and chlorophyll content was decreased in a similar way as observed for those of uninoculated plants. However, substantial increase in leghaemoglobin and chlorophyll content was observed in inoculated greengram plants compared to the uninoculated plants treated with the same concentration of pesticides. For example, *P. aeruginosa* strain PS1 with recommended dose of tebuconazole increased leghaemoglobin and chlorophyll content by 14% and 12% respectively, compared to the uninoculated plants treated with same dose of tebuconazole (Table 86).

4.7.5.3 Nutrient uptake and grain attributes

Nitrogen and phosphorus content, seed yield and grain protein measured at harvesting decreased consistently with increasing the dose rate of each pesticide both in the presence and absence of the inoculant. Without inoculant, the percent decrease in root N, shoot N, root P, shoot P, SY and GP in the presence of quizalafop-p-ethyl was 45, 44, 52, 37, 63 and 12 (Table 82); 17, 16, 15, 20, 29 and 4 for clodinafop (Table 83); 34, 22, 23, 14, 38 and 5 for fipronil (Table 84); 37, 32, 38, 25, 40 and 7 for pyriproxyfen (Table 85) and 25, 30, 38, 34, 49 and 8 for tebuconazole (Table 86), respectively, at 3X, compared to uninoculated control plants. Moreover, when inoculant strain PS1 was used with pesticide, similar decreasing trend for N, P,

SY and GP was observed but the toxicity of the pesticides considerably decreased in comparison to uninoculated plants. Furthermore, the inoculant strain significantly ($P \leq 0.05$) increased the root N, shoot N, root P, shoot P, SY and GP at all concentration of pesticides. For example, the phosphate solubilizing bacterial inoculant when used with 3X of clodinafop, significantly ($P \leq 0.05$) increased the root N, shoot N, shoot P and SY by 27, 38, 34 and 83%, respectively, when compared to the treatment having the same dose of clodinafop but devoid of inoculant (Table 83). The two-way ANOVA of the measured parameters of mungbean plants revealed that the individual effects of PSB inoculation and pesticides and their interaction (inoculation × pesticides) were significant ($P \leq 0.05$).

While comparing the effect of 3X of all pesticides on the performance of uninoculated legumes raised in sandy clay loam soils, it was found that the phytotoxicity of pesticides on all tested crops was variable and dose dependent decrease in plant growth was observed. Quizalafop-p-ethyl, among pesticides, affected most adversely the symbiosis and seed attributes of all legume crops under study. Furthermore, quizalafop-p-ethyl at three times of recommended dose decreased maximally the nodule numbers (100%) for both pea and lentil, seed yield (80%) for lentil and grain protein (33%) for chickpea over their respective controls. Similarly, in case of nodulation, clodinafop at 3X, showed maximum toxicity to pea plants by decreasing nodule numbers by 60% while in case of seed yield and grain protein, it exhibited greatest phytotoxicity to chickpea plants and decreased yield upto 38% relative to respective controls. Of insecticides, fipronil at three times more of recommended dose, showed most inhibitory effect on nodule numbers (67% decrease) of pea and on the seed yield (52% decrease) of chickpea plants compared to their respective controls. Moreover, pyriproxyfen (3X) deranged symbiosis and significantly ($P \leq 0.05$) decreased nodulation (63% decline in nodule number) of pea plants and both seed yield (60% decrease) and protein content (18% reduction) of chickpea plants. Furthermore, at the same dose rate, tebuconazole showed a maximum reduction in nodule numbers (100%) each for pea and lentil plants while seed yield and grain protein were reduced by 70 and 24%, respectively (Table 87).

Tables

Table 13 Microbial diversity in different soil samples collected from experimental fields of Faculty of Agricultural Sciences, Aligarh Muslim University, Aligarh, India

Sampling site	Microbial populations (colony forming units/g soil)			
	Bacteria (x 10^5)	Fungi (x 10^4)	Phosphate solubilizers (x 10^5)	
			Bacteria	Fungi
Chickpea field	321	13	6	-
Greengram field	286	21	2	0.2
Lentil field	353	12	3	0.2
Pea field	311	16	4	0.3
Mustard field	435	19	6	0.3

Each value is a mean of three independent replicates

Table 14 Morphological and biochemical characteristics of plant growth promoting rhizobacteria

Characteristics	Rhizobial groups (N=35)			Phosphate solubilizers (N=18)			
	MRC	MRL /MRP	MRM	PSB (N=2)	PSB (N=1)	PSB (N=14)	PSB (N=1)
Morphological							
Gram reaction	G-ve	G-ve	G-ve	G-ve	G-ve	G+ve	G-ve
Cell shape	Short rods	Short rods	Short rods	Short rods	rods	rods	rods
Colony morphology	Transparent, circular and mucoid	Transparent, circular and mucoid	Transparent, circular and mucoid	Mucoid, smooth margin	Mucoid, smooth margin	Mucoid, serrate margin	Mucoid, smooth margin
Biochemical tests							
Nitrate	31	23/20	26	11	6	76	6
Methyl red	-	-	-	-	6	76	6
Catalase	31	23/20	26	11	-	76	-
Citrate	31	23/20	26	11	6	76	6
Voges Proskauer	-	-	-	-	6	76	6
Indole	-	-	-	11	-	76	6
Hydrolysis							
Gelatin	-	-	-	11	-	76	-
Starch	31	23/20	26	-	-	76	-
Carbohydrate utilization							
Glucose	31	23/20	26	11	6	76	6
Sucrose	31	23/20	26	-	6	76	6
Mannitol	31	23/20	26	-	-	76	-
Presumptive identification	*Mesorhizobium*	*Rhizobium*	*Bradyrhizobium*	*Pseudomonas*	*Enterobacter*	*Bacillus*	*Klebsiella*

MRC, MRL, MRP and MRM indicates the rhizobial strains isolated from the nodules produced on the root systems of chickpea, lentil, pea and greengram plants, respectively. PSB indicates phosphate solubilizing bacteria isolated from rhizosphere soils of mustard. N indicates the total number of isolates used. Values indicate the percent of bacterial strains showing positive reaction to each biochemical test. - indicates a negative reaction

Table 15 Plant growth promoting (PGP) activity based typing of *Mesorhizobium* strains (N=11) isolated from chickpea nodules

PGP groups	Isolate designation	Production of				Exo-polysaccharides (EPS)	Phosphate solubilization	Activity profile
		Ammonia	HCN	Siderophore on CAS	IAA			
I	MRC1, MRC4, MRC7, MRC10	4 (36)	4 (36)	4 (36)	4 (36)	4 (36)	-	A, H, S, I, EPS
II	MRC3, MRC9, MRC12,	3 (27)	3 (27)	-	3 (27)	-	-	A, H, I
III	MRC5, MRC6, MRC11,	3 (27)	-	-	3 (27)	-	-	A, I
IV	MRC14	-	-	-	1 (9)	-	-	I
Total number of PGP positive isolates		10 (90)	7 (63)	4 (36)	11 (100)	4 (36)	-	

In this and succeeding tables, A=Ammonia; H= HCN; S= Siderophore; I= IAA, EPS= Exo-polysaccharides; values indicate the percent of strains positive to each reaction; CAS indicates Chrome azurol S agar; in this and succeeding tables values in parenthesis indicate the percent value.

Table 16 Plant growth promoting (PGP) activity based typing of *Rhizobium* strains (N=7) isolated from pea nodules

PGP groups	Isolate designation	Production of				Exo-polysaccharides (EPS)	Phosphate solubilization	Activity profile
		Ammonia	HCN	Siderophore on CAS	IAA			
I	MRP1, MRP4 MRP7	3 (43)	3 (43)	3 (43)	3 (43)	3 (43)	-	A, H, S, I, EPS
II	MRP2	-	1 (14)	-	1 (14)	-	-	H, I
III	MRP3, MRP5, MRP6	3 (43)	3 (43)	-	3 (43)	-	-	A, H, I
Total number of PGP positive isolates		6 (86)	7 (100)	3 (43)	7 (100)	3 (43)	-	

Table 17 Plant growth promoting (PGP) activity based typing of *Bradyrhizobium* strains (N=9) isolated from greengram nodules

PGP groups	Isolate designation	Production of				Exo-polysaccharides (EPS)	Phosphate solubilization	Activity profile
		Ammonia	HCN	Siderophore on CAS	IAA			
I	MRM3, MRM6, MRM8	3 (33)	3 (33)	3 (33)	3 (33)	3 (33)	-	A, H, S, I, EPS
II	MRRM1, MRRM2, MRM5, MRM7, MRM9,	5 (56)	-	5 (56)	5 (56)	-	-	A, S, I
III	MRM4	1 (11)	-	-	1 (11)	-	-	A, I
Total number of PGP positive isolates		9 (100)	3 (33)	8 (88)	9 (100)	3 (33)	-	

Table 18 Plant growth promoting (PGP) activity based typing of *Rhizobium* strains (N=8) isolated from lentil nodules

PGP groups	Isolate designation	Production of				Exo-polysaccharides (EPS)	Phosphate solubilization	Activity profile
		Ammonia	HCN	Siderophore on CAS	IAA			
I	MRL1, MRL3, MRL6.	3 (37)	3 (37)	3 (37)	3 (37)	3 (37)	-	A, H, S, I, EPS
II	MRL7	1 (13)	-	1 (13)	1 (13)	-	-	A, S, I
III	MRL2, MRL4, MRL8	3 (37)	3 (37)	-	3 (37)	-	-	A, H, I
IV	MRL5	1 (13)	-	-	1 (13)	-	-	A, I
Total number of PGP positive isolates		8 (100)	6 (75)	4 (50)	8 (100)	3 (37)	-	

Table 19 Plant growth promoting (PGP) activity based typing of phosphate solubilizing bacteria (N=18) isolated from mustard rhizosphere

PGP groups	Isolate designation	Production of				Exo-polysaccharides (EPS)	Phosphate solubilization	Activity profile
		Ammonia	HCN	Siderophore on CAS	IAA			
I	PS1, PS2, PS9, PS19	4(22)	4(22)	4(22)	4(22)	4(22)	4(22)	A, H, S, I, P, EPS
II	PS5, PS7, PS12, PS14, PS22, PS23	6(33)	-	6(33)	6(33)	6(33)	6(33)	A, S, I, P,E
III	PS3, PS4, PS10, PS16, PS20	5(28)	5(28)	-	5(28)	5(28)	5(28)	A, H, I, P,E
IV	PS6, PS17, PS21	3(17)	-	-	3(17)	3(17)	3(17)	A, I, P,E
Total number of PGP positive isolates		18(100)	9(50)	10(56)	18(100)	18(100)	18(100)	

Table 20 Plant growth promoting activities of *Mesorhizobium* species (N= 11) isolated from chickpea nodules

Rhizobial strains	Plant growth promoting activities						
	Siderophores			IAA (µg/ml)	EPS (µg/ml)	Ammonia	HCN[e]
	Zone on CAS Agar[a] (mm)	SA[b] (µg/ml)	2,3,DHBA[c] (µg/ml)	100T[d]			
MRC1	11±1	30±1.7	17±1.3	18.±1.5	16±1.5	+	+
MRC3	-	-	-	15±2.2	-	+	+
MRC4	12±1	35±1.5	19±1.7	44±2.4	21±2.3	+	+
MRC5	-	-	-	43±2.5	-	+	-
MRC6	-	-	-	31±2.3	-	+	-
MRC7	10±1	25±1.0	18±1.4	18±1.3	10±1.1	+	+
MRC9	-	-	-	16±2.4	-	+	+
MRC10	9±1	21±1.2	17±1.1	14±2.5	8±0.7	+	+
MRC11	-	-	-	17±1.9	-	+	-
MRC12	-	-	-	17±1.7	-	+	+
MRC14	-	-	-	15±1.5	-	-	-

In this and succeeding tables [a]Chrome azurol S agar; [b]Salicylic acid; [c]2,3 Dihydroxy benzoic acid; [d] Tryptophan concentration (µg ml^{-1}); [e]Hydrogen cyanide; + indicates positive reaction; - indicates no reaction. Values in this and succeeding tables indicate mean ± S.D of three replicates.

Table 21 Plant growth promoting activities of *Rhizobium* species (N= 7) isolated from pea nodules

Rhizobial strains	Plant growth promoting activities						
	Siderophores			IAA (µg/ml)	EPS (µg/ml)	Ammonia	HCN
	Zone on CAS agar (mm)	SA (µg/ml)	2,3,DHBA (µg/ml)	100T			
MRP1	11±1	32±1.5	22±1.1	32±1.6	20±1.3	+	+
MRP2	-	-	-	19±2.1	-	-	+
MRP3	-	-	-	28±2.3	-	+	+
MRP4	10±1	29±1.9	18±0.9	17±0.8	15±1.7	+	+
MRP5	-	-	-	27±1.3	-	+	+
MRP6	-	-	-	25±2.6	-	+	+
MRP7	11±2	25±1.2	14±1.3	23±1.4	18±1.6	+	+

Table 22 Plant growth promoting activities of *Bradyrhizobium* species (N= 9) isolated from nodules of greengram plants

Rhizobial strains	Plant growth promoting activities						
	Siderophores			IAA (µg/ml)	EPS (µg/ml)	Ammonia	HCN
	Zone on CAS agar (mm)	SA (µg/ml)	2,3,DHBA (µg/ml)	100T			
MRM1	-	-	-	17±2.2	-	+	-
MRM2	-	-	-	29±1.1	-	+	-
MRM3	10±1	30±2.3	15±1.2	17±1.3	13±1.4	+	+
MRM4	-	-	-	23±1.4	-	+	-
MRM5	-	-	-	34±1.7	-	+	-
MRM6	13±1	32±1.8	18±1.3	38±1.8	21±1.6	+	+
MRM7	-	-	-	15±2.4	-	+	-
MRM8	11±1	28±1.5	16±2.1	18±1.2	14±1.3	+	+
MRM9	-	-	-	21±1.6	-	+	-

Table 23 Plant growth promoting activities of *Rhizobium* species (N= 8) recovered from nodules of lentil plants

Rhizobial strains	Plant growth promoting activities						
	Siderophores			IAA (µg/ml)	EPS (µg/ml)	Ammonia	HCN
	Zone on CAS agar (mm)	SA (µg/ml)	2,3,DHBA (µg/ml)	100T			
MRL1	10±2	26±2.4	18±1.2	21±1.5	13±0.8	+	+
MRL2	-	-	-	15±2.3	-	+	+
MRL3	12±1	29±1.6	21±1.4	37±1.7	18±1.6	+	+
MRL4	-	-	-	23±1.6	-	+	+
MRL5	-	-	-	30±1.1	-	+	-
MRL6	10±1	27±1.2	17±1.5	18±2.2	11±1.0	+	+
MRL7	10±2	25±1.7	15±1.4	15±1.3	-	+	-
MRL8	-	-	-	17±1.4	-	+	+

Table 24 Plant growth promoting potentials of phosphate solubilizing bacteria (N= 18) isolated from rhizospheric soils

Bacterial strains	Plant growth promoting activities						
	Siderophores			IAA (µg/ml)	EPS (µg/ml)	Ammonia	HCN
	Zone on CAS agar (mm)	SA (µg/ml)	2,3,DHBA (µg/ml)	100T			
PS1	15±0.8	41±1.2	21±0.3	39±2.6	18±2.1	+	+
PS2	13±0.6	24±1.1	9±0.2	32±2.1	16±1.4	+	+
PS3	-	-	-	21±1.6	8±0.5	+	+
PS4	-	-	-	17±1.3	9±0.3	+	+
PS5	12±1.0	11±0.9	5±0.3	22±1.4	13±1.2	+	-
PS6	-	-	-	20±1.5	8±0.3	+	-
PS7	11±0.7	21±0.7	6±0.9	26±2.1	7±0.2	+	-
PS9	14±0.8	41±0.4	17±1.2	34±1.7	17±1.1	+	+
PS10	-	-	-	14±0.9	16±1.6	+	+
PS12	9±0.6	23±0.6	9±0.5	11±1.2	10±1.2	+	-
PS14	8±0.8	20±0.3	4±0.3	15±1.3	12±1.4	+	-
PS16	-	-	-	23±2.4	8±0.8	+	+
PS17	-	-	-	23±1.7	7±0.6	+	-
PS19	14±0.7	47±0.5	10±0.3	42±2.7	18±1.4	+	+
PS20	-	-	-	27±1.5	12±1.3	+	+
PS21	-	-	-	13±1.1	12±0.8	+	-
PS22	8±0.5	14±0.4	6±0.5	12±1.3	9±1.1	+	-
PS23	10±0.9	15±0.6	4±0.4	9±0.8	9±0.9	+	-

Table 25 Plant growth promoting activities of *Mesorhizobium* strain MRC4 in the presence of varying concentrations of herbicides, insecticides and fungicides

Pesticides		Dose rate (µg /l)	Plant growth promoting activities						
			Siderophores			IAA (µg /ml)	EPS (µg /ml)	Ammonia	HCN
			Zone on CAS agar (mm)	SA (µg/ml)	DHBA (µg/ml)	100T			
Herbicides	Quizalafop-p-ethyl	40	11±1	25±1.2	15±1.5	19±1.5	21±1.8	+	+
		80	10±1	22±1.4	13±1.2	15±1.6	21±2.1	+	+
		120	10±1	19±1.5	10±1.2	11±1.0	23±2.3	+	+
	Clodinafop	400	12±1	32±1.6	17±1.5	33±1.5	22±2.4	+	+
		800	10±1	30±1.1	15±1.3	23±1.8	25±1.9	+	+
		1200	10±1	27±1.2	12±1.2	18±1.3	27±1.8	+	+
	Metribuzin	850	11±1	33±1.4	18±1.5	41±2.1	22±2.5	+	+
		1700	10±2	32±1.3	17±1.1	39±1.9	24±2.2	+	+
		2550	10±2	30±1.1	18±1.4	37±1.7	27±1.8	+	+
	Glyphosate	1444	12±1	32±1.6	16±1.2	38±2.3	23±1.6	+	+
		2888	11±1	31±1.2	15±1.5	36±2.3	25±2.3	+	+
		4332	11±1	29±1.3	13±1.3	33±1.5	26±2.6	+	+
Insecticides	Fipronil	200	12±1	30±1.2	18±1.3	33±1.2	22±2.3	+	+
		400	12±1	28±1.1	15±1.4	27±1.3	24±2.1	+	+
		600	11±2	27±1.3	13±1.2	22±1.4	26±1.8	+	+
	Pyriproxyfen	1300	12±1	28±1.2	16±1.6	29±2.1	23±1.9	+	+
		2600	12±1	25±1.1	15±1.1	21±2.2	24±1.6	+	+
		3900	11±2	21±1.3	12±1.0	17±1.3	26±2.7	+	+
	Imidacloprid	100	12±2	29±1.5	18±1.2	40±1.2	26±1.7	+	+
		200	11±1	27±1.5	15±1.3	37±1.5	28±2.5	+	+
		300	11±1	23±1.0	15±1.5	35±1.5	29±2,3	+	+
	Thiamethoxam	25	12±1	33±1.4	13±1.2	42±2.0	24±2.1	+	+
		50	12±1	26±1.3	10±1.1	39±1.6	25±1.9	+	+
		75	12±1	23±1.4	8±1.3	36±1.2	27±2.4	+	+
Fungicides	Tebuconazole	100	9±1	26±1.0	13±1.2	17±1.4	22±1.6	+	+
		200	8±2	23±1.2	10±1.3	14±1.2	23±2.5	+	+
		300	8±2	21±1.1	8±1.2	11±1.3	25±1.6	+	+
	Hexaconazole	40	9±1	26±1.1	14±1.4	28±2.8	23±1.5	+	+
		80	8±1	24±1.3	12±1.5	25±1.9	26±2.3	+	+
		120	8±2	21±1.2	10±1.2	21±1.5	28±1.3	+	+
	Metalaxyl	1500	12±1	28±1.2	16±1.4	34±2.0	23±1.5	+	+
		3000	10±1	25±1.2	15±1.2	31±2.3	25±1.6	+	+
		4500	9±2	22±1.1	14±1.4	29±2.2	26±1.4	+	+
	Kitazin	96	12±1	31±1.2	17±1.3	37±2.5	22±1.6	+	+
		192	10±2	28±1.3	15±1.4	35±2.1	24±2.4	+	+
		288	9±2	26±1.1	14±1.3	32±1.8	27±2.2	+	+
Control (without pesticide)			12±1	35±1.5	19±1.7	44±2.4	21±2.3	+	+

In this and succeeding tables, values indicate mean ± S.D of three replicates.

Table 26 Plant growth promoting activities of *Rhizobium* strain MRP1 in the presence of varying concentrations of herbicides, insecticides and fungicides

Pesticides		Dose rate (µg /l)	Plant growth promoting activities						
			Siderophores			IAA (µg /ml)	EPS (µg /ml)	Ammonia	HCN
			Zone on CAS agar (mm)	SA (µg /ml)	DHBA (µg /ml)	100T			
Herbicides	Quizalafop-p-ethyl	40	10±1	22±1.4	15±1.2	23±1.3	20±1.2	+	+
		80	10±1	19±1.2	14±1.1	21±1.2	22±1.3	+	+
		120	9±1	14±1.3	10±1.4	18±1.1	23±1.4	+	+
	Clodinafop	400	11±1	28±1.1	20±1.6	30±1.3	21±1.2	+	+
		800	10±2	25±1.2	18±1.4	28±1.1	23±1.1	+	+
		1200	10±1	21±1.1	15±1.3	25±1.7	26±1.2	+	+
	Metribuzin	850	11±2	31±1.5	20±1.2	30±1.8	22±2.1	+	+
		1700	11±2	29±1.6	18±1.7	27±1.4	24±1.3	+	+
		2550	11±1	27±1.3	16±1.6	26±2.1	27±2.5	+	+
	Glyphosate	1444	11±2	30±1.7	19±1.24	28±1.8	22±1.7	+	+
		2888	11±1	28±1.3	17±1.3	25±1.9	25±2.4	+	+
		4332	11±1	26±1.2	15±1.2	23±1.4	28±1.2	+	+
Insecticides	Fipronil	200	11±1	30±1.1	18±1.2	25±1.5	21±1.1	+	+
		400	11±1	28±1.3	17±1.3	24±1.4	25±1.8	+	+
		600	10±1	25±1.4	15±1.6	21±1.6	25±2.2	+	+
	Pyriproxyfen	1300	11±1	26±1.1	16±1.4	29±1.9	22±2.1	+	+
		2600	11±1	23±1.7	14±1.3	26±2.4	23±1.7	+	+
		3900	10±1	21±1.4	12±1.3	23±1.8	26±1.8	+	+
	Imidacloprid	100	11±2	28±1.4	20±1.2	29±1.3	21±1.2	+	+
		200	10±2	26±1.3	18±1.1	28±1.6	24±2.3	+	+
		300	10±1	24±1.2	16±1.5	26±1.7	26±2.4	+	+
	Thiamethoxam	25	11±2	30±1.1	21±1.3	31±2.3	20±2.3	+	+
		50	11±1	28±1.2	18±1.7	30±2.3	21±2.2	+	+
		75	11±1	23±1.6	16±1.2	28±1.5	23±1.1	+	+
Fungicides	Tebuconazole	100	10±1	24±1.2	15±1.2	22±1.2	22±1.1	+	+
		200	10±1	20±1.3	10±1.1	17±1.5	24±1.3	+	+
		300	9±1	18±1.0	9±1.1	16±1.6	25±2.2	+	+
	Hexaconazole	40	11±2	26±1.3	14±1.5	28±2.4	22±1.2	+	+
		80	10±1	23±1.5	12±1.3	25±1.6	24±2.5	+	+
		120	9±1	20±1.1	10±1.5	21±1.4	25±1.6	+	+
	Metalaxyl	1500	11±1	28±1.5	19±1.2	28±1.7	22±1.9	+	+
		3000	11±2	27±1.6	18±1.5	26±2.3	23±2.2	+	+
		4500	10±1	25±1.2	16±1.3	23±1.9	25±2.2	+	+
	Kitazin	96	11±1	29±1.7	20±1.2	30±1.8	20±1.5	+	+
		192	11±1	28±1.3	19±1.5	27±1.6	21±2.2	+	+
		288	11±1	26±1.0	17±1.5	25±2.1	23±1.8	+	+
Control (without pesticide)			11±1	32±1.5	22±1.1	32±1.6	20±1.3	+	+

Table 27 Plant growth promoting activities of *Bradyrhizobium* strain MRM6 under the influence of varying concentrations of herbicides, insecticides and fungicides

Pesticides		Dose rate (µg /l)	Plant growth promoting activities						
			Siderophores			IAA (µg /ml)	EPS (µg /ml)	Ammonia	HCN
			Zone on CAS agar (mm)	SA (µg /ml)	DHBA (µg /ml)	100T			
Herbicides	Quizalafop-p-ethyl	40	12±1.2	21±2.2	14±2.1	7±1.4	22±2.2	+	+
		80	11±1.1	19±1.1	8±1.2	4±1.5	24±1.3	+	+
		120	10±1.3	12±1.4	5±0.7	3±1.6	25±0.4	+	+
	Clodinafop	400	12±1.0	21±2.1	15±1.5	17±2.2	21±1.2	+	+
		800	12±1.2	19±1.3	11±2.2	9±1.3	23±1.1	+	+
		1200	11±0.8	14±2.0	9±1.7	7±1.5	24±1.2	+	+
	Metribuzin	850	13±1.5	28±1.2	16±0.9	30±2.6	21±1.1	+	+
		1700	13±1.0	27±2.1	15±1.3	27±1.5	22±2.3	+	+
		2550	13±1.2	24±1.5	12±1.2	26±2.0	25±1.4	+	+
	Glyphosate	1444	13±1.6	29±2.2	16±1.4	28±2.5	23±0.9	+	+
		2888	13±1.4	26±1.7	14±1.1	25±1.4	26±1.5	+	+
		4332	13±0.8	23±2.4	13±1.1	23±1.5	29±1.6	+	+
Insecticides	Fipronil	200	12±1.5	30±1.6	18±0.8	27±2.3	22±1.3	+	+
		400	11±0.9	28±1.2	17±1.4	15±1.6	23±2.4	+	+
		600	10±0.8	25±2.3	15±1.5	12±1.3	26±1.4	+	+
	Pyriproxyfen	1300	12±1.4	26±1.5	16±0.9	16±1.4	22±1.5	+	+
		2600	12±0.9	23±2.4	14±1.4	10±1.5	25±2.2	+	+
		3900	11±0.7	21±1.2	12±0.9	7±1.3	26±2.5	+	+
	Imidacloprid	100	13±1.4	28±1.5	16±0.8	32±2.2	21±2.3	+	+
		200	13±1.3	25±2.2	14±2.0	30±1.4	23±2.4	+	+
		300	12±0.6	23±1.5	12±1.3	27±1.5	25±1.5	+	+
	Thiamethoxam	25	13±1.3	30±1.3	17±2.1	35±1.2	22±2.1	+	+
		50	12±1.2	29±1.2	16±1.8	33±2.2	23±1.5	+	+
		75	11±1.0	27±1.5	15±1.3	31±1.7	26±2.3	+	+
	Tebuconazole	100	12±0.5	24±1.9	15±1.1	7±1.6	23±2.3	+	+
Fungicides		200	11±0.6	21±1.4	13±0.7	6±1.8	25±0.8	+	+
		300	10±0.7	18±1.5	10±0.8	4±1.4	27±1.4	+	+
	Hexaconazole	40	12±1.0	26±2.0	16±2.4	28±2.1	22±2.1	+	+
		80	11±1.0	23±2.3	14±1.7	24±1.6	25±1.6	+	+
		120	11±0.8	20±1.4	11±0.9	22±1.4	27±2.3	+	+
	Metalaxyl	1500	12±1.4	28±1.3	17±1.3	35±2.3	22±0.9	+	+
		3000	12±1.2	27±2.2	15±2.1	32±1.4	24±1.4	+	+
		4500	11±0.9	25±1.4	13±1.7	29±1.6	26±1.5	+	+
	Kitazin	96	13±1.0	30±2.5	17±2.2	36±2.4	21±1.6	+	+
		192	13±1.2	28±2.1	16±2.6	34±2.1	22±1.3	+	+
		288	13±1.0	26±1.5	14±1.8	31±1.7	24±2.4	+	+
Control (without pesticide)			13±1	32±1.8	18±1.3	38±1.8	21±1.6	+	+

Table 28 Plant growth promoting activities of *Rhizobium* strain MRL3 isolated from lentil nodules in the presence of varying concentrations of herbicides, insecticides and fungicides

Pesticides		Dose rate (µg /l)	Plant growth promoting activities						
			Siderophores			IAA (µg /ml)	EPS (µg/ml)	Ammonia	HCN
			Zone on CAS agar (mm)	SA (µg /ml)	DHBA (µg /ml)	100T			
Herbicides	Quizalafop-p-ethyl	40	11±0.3	21±1.4	16±1.7	27±1.2	20±1.3	+	+
		80	10±0.5	17±1.0	10±1.4	23±1.6	21±1.4	+	+
		120	9±0.3	15±1.2	9±1.3	20±0.8	24±1.4	+	+
	Clodinafop	400	11±1.0	25±1.5	17±1.6	33±0.9	19±1.5	+	+
		800	10±1.1	22±1.2	14±1.4	27±1.0	20±1.2	+	+
		1200	9±0.7	17±1.6	10±0.9	22±1.3	22±1.0	+	+
	Metribuzin	850	12±1.4	29±2.2	20±0.8	35±1.4	18±1.2	+	+
		1700	12±1.1	28±1.3	19±1.0	32±1.3	19±1.5	+	+
		2550	11±1.0	26±1.2	18±1.0	30±1.0	21±1.6	+	+
	Glyphosate	1444	12±0.6	27±1.2	18±1.2	32±1.7	20±1.4	+	+
		2888	12±0.5	25±1.5	17±0.6	29±1.5	22±1.1	+	+
		4332	11±0.5	22±1.7	15±2.1	27±0.7	24±1.4	+	+
Insecticides	Fipronil	200	11±0.5	27±1.4	18±0.7	31±1.1	19±1.0	+	+
		400	11±0.6	21±1.5	16±1.2	28±1.4	22±1.4	+	+
		600	10±0.7	18±1.8	13±1.7	24±1.5	23±1.5	+	+
	Pyriproxyfen	1300	11±1.5	25±1.4	14±1.2	28±1.4	19±1.4	+	+
		2600	11±1.3	24±1.2	12±1.4	23±1.8	21±2.0	+	+
		3900	10±1.3	21±2.2	9±1.5	20±1.4	24±1.5	+	+
	Imidacloprid	100	12±0.8	25±2.5	16±1.8	26±1.6	23±1.1	+	+
		200	11±0.7	23±1.3	15±1.8	25±1.2	25±1.6	+	+
		300	11±0.6	21±1.7	13±1.5	23±1.2	26±1.5	+	+
	Thiamethoxam	25	11±1.4	25±1.4	17±1.5	28±1.3	22±1.2	+	+
		50	10±0.9	24±2.2	15±1.6	27±1.1	24±1.2	+	+
		75	10±0.6	22±1.2	14±1.5	25±1.5	25±1.5	+	+
Fungicides	Tebuconazole	100	11±0.7	20±1.5	14±1.7	21±1.4	20±1.4	+	+
		200	10±1.2	16±2.0	12±1.4	17±1.5	20±1.1	+	+
		300	9±0.6	14±1.3	10±1.4	15±1.2	22±1.5	+	+
	Hexaconazole	40	12±1.1	24±1.5	16±1.6	25±2.1	24±1.6	+	+
		80	11±1.2	22±1.5	14±1.2	24±1.6	26±1.2	+	+
		120	10±0.6	21±1.7	12±0.5	21±1.2	27±0.8	+	+
	Metalaxyl	1500	12±1.2	26±2.1	18±1.2	30±2.5	21±2.3	+	+
		3000	12±1.5	25±1.2	16±1.4	29±1.7	23±1.6	+	+
		4500	11±1.3	23±1.5	14±1.5	27±1.4	25±1.9	+	+
	Kitazin	96	12±0,8	28±1.4	19±1.4	34±1.5	19±2.2	+	+
		192	12±1.0	27±1.6	18±1.5	32±2.0	21±1.6	+	+
		288	12±1.2	25±1.8	17±0.7	30±1.2	22±1.5	+	+
Control (without pesticide)			12±1	29±1.6	21±1.4	37±1.7	18±1.6	+	+

Table 29 Plant growth promoting activities of phosphate solubilizing bacterium *Pseudomonas aeruginosa* strain PS1 grown in the presence of varying concentrations of herbicides, insecticides and fungicides

Pesticides		Dose rate (µg /l)	Plant growth promoting activities					Ammonia	HCN
			Siderophores			IAA (µg /ml)	EPS (µg /ml)		
			Zone on CAS agar (mm)	SA (µg /ml)	DHBA (µg /ml)	100T			
Herbicides	Quizalafop-p-ethyl	40	12±1.2	38±2.4	19±1.2	9±1.1	21±1.4	+	+
		80	12±1.4	32±1.2	15±1.3	5±0.3	23±1.5	+	+
		120	11±0.8	27±1.3	11±1.5	4±0.5	25±2.1	+	+
	Clodinafop	400	14±1.5	39±2.5	10±1.2	25±1.4	22±1.3	+	+
		800	13±1.2	34±1.8	8±0.5	13±1.6	24±1.7	+	+
		1200	13±1.6	29±1.8	6±0.7	9±0.7	25±1.1	+	+
	Metribuzin	850	15±2.4	38±1.6	15±1.2	25±1.2	20±1.5	+	+
		1700	15±1.3	35±2.2	9±0.5	21±0.2	22±1.2	+	+
		2550	14±2.2	31±2.2	7±0.6	18±0.5	24±1.3	+	+
	Glyphosate	1444	15±2.2	37±1.1	12±0.9	24±1.7	20±1.2	+	+
		2888	14±1.1	32±2.1	10±1.2	14±1.6	23±1.2	+	+
		4332	14±2.1	27±1.3	8±0.6	12±1.2	25±1.5	+	+
Insecticides	Fipronil	200	14±1.1	37±1.1	20±1.3	18±0.3	19±1.6	+	+
		400	13±1.3	31±1.2	14±1.2	14±0.6	24±1.2	+	+
		600	12±0.9	24±1.3	9±1.1	12±1.5	26±1.3	+	+
	Pyriproxyfen	1300	14±1.2	36±2.1	12±1.4	15±1.4	18±1.3	+	+
		2600	13±1.5	30±2.0	8±0.5	9±0.3	25±1.7	+	+
		3900	12±1.5	22±1.4	6±0.7	6±0.5	27±2.1	+	+
	Imidacloprid	100	14±1.6	30±2.1	8±0.6	19±2.2	20±1.3	+	+
		200	13±1.3	24±2.0	6±0.3	12±1.8	24±1.8	+	+
		300	13±1.2	21±1.3	4±0.5	9±0.9	25±1.3	+	+
	Thiamethoxam	25	14±1.6	30±1.3	9±0.4	21±1.2	21±1.4	+	+
		50	14±1.5	26±2.1	7±0.8	16±1.1	22±1.1	+	+
		75	13±1.3	22±1.6	5±0.4	12±1.5	24±1.3	+	+
Fungicides	Tebuconazole	100	14±1.1	27±2.4	8±0.6	9±0.8	19±1.5	+	+
		200	13±1.5	23±1.2	7±0.4	5±0.6	20±1.5	+	+
		300	12±0.9	20±1.4	5±0.6	3±0.9	23±1.6	+	+
	Hexaconazole	40	14±1.2	36±2.2	13±1.1	18±1.7	22±1.2	+	+
		80	13±1.6	30±1.5	10±0.8	11±0.6	24±1.6	+	+
		120	13±1.4	26±1.2	6±0.5	8±0.9	28±1.7	+	+
	Metalaxyl	1500	15±2.0	32±2.0	10±0.9	21±1.8	18±1.9	+	+
		3000	14±1.2	23±1.4	7±0.5	13±1.4	22±2.0	+	+
		4500	14±2.1	19±1.2	5±0.7	9±1.1	26±1.2	+	+
	Kitazin	96	15±2.4	35±2.1	15±1.0	25±2.0	18±1.7	+	+
		192	15±2.2	29±1.4	9±0.6	17±1.3	22±1.5	+	+
		288	14±1.3	26±1.6	7±0.8	14±1.6	24±2.3	+	+
Control (without pesticide)			15±2.0	41±2.2	21±1.5	39±2.3	18±2.1	+	+

Table 30 Plant growth promoting activities of phosphate solubilizing bacterium *Enterobacter asburiae* strain PS2 in the presence of varying concentrations of herbicides, insecticides and fungicides

Pesticides		Dose rate (µg /l)	Plant growth promoting activities					Ammonia	HCN
			Siderophores			IAA (µg /ml)	EPS (µg /ml)		
			Zone on CAS agar (mm)	SA (µg /ml)	DHBA (µg /ml)	100T			
Herbicides	Quizalafop-p-ethyl	40	11±1.0	21±1.3	5±0.5	11±1.2	11±1.0	+	+
		80	10±1.0	14±1.6	4±0.7	4±0.5	10±1.0	+	+
		120	9±1.0	9±1.5	2±0.4	3±0.6	9±1.0	+	-
	Clodinafop	400	12±1.0	23±2.5	5±0.4	15±1.5	12±1.0	+	-
		800	11±1.0	21±1.3	4±0.6	9±0.4	11±1.0	+	-
		1200	10±1.5	15±1.2	3±0.7	7±0.3	10±1.5	+	-
	Metribuzin	850	12±1.0	20±1.1	7±0.5	21±1.3	12±1.0	+	-
		1700	12±1.3	19±2.3	5±0.8	15±1.4	12±1.3	+	-
		2550	11±1.0	16±2.2	4±0.9	13±1.5	11±1.0	+	-
	Glyphosate	1444	12±1.0	18±1.4	6±0.6	19±1.6	12±1.0	+	-
		2888	11±1.0	15±1.5	5±0.7	13±1.5	11±1.0	+	+
		4332	11±1.0	13±1.2	3±0.4	10±1.4	11±1.0	+	-
Insecticides	Fipronil	200	11±1.0	25±1.8	5±0.5	21±1.5	11±1.0	+	-
		400	10±1.0	14±1.4	4±0.8	14±1.6	10±1.0	+	-
		600	9±0.5	11±1.4	2±0.3	11±1.7	9±0.5	+	-
	Pyriproxyfen	1300	10±1.5	23±1.5	5±0.6	26±1.8	10±1.5	+	-
		2600	9±1.0	21±2.5	4±0.3	13±1.7	9±1.0	+	-
		3900	8±1.0	18±1.5	3±0.4	9±1.8	8±1.0	+	-
	Imidacloprid	100	12±1.0	14±1.4	5±0.5	16±1.9	12±1.0	+	-
		200	11±1.0	10±2.2	3±0.7	8±0.39	11±1.0	+	-
		300	10±1.0	8±1.3	2±0.6	5±0.5	10±1.0	+	-
	Thiamethoxam	25	12±1.4	16±1.3	6±0.8	16±1.4	12±1.4	+	+
		50	11±1.5	11±1.2	4±0.4	13±0.7	11±1.5	+	+
		75	11±1.0	8±1.0	2±0.8	10±0.2	11±1.0	+	+
Fungicides	Tebuconazole	100	10±1.0	15±1.2	6±0.4	8±0.7	10±1.0	+	-
		200	9±1.0	14±1.1	4±0.3	4±0.6	9±1.0	+	-
		300	8±1.0	12±1.23	3±0.6	2±0.5	8±1.0	+	-
	Hexaconazole	40	11±1.2	13±2.2	4±0.5	14±1.6	11±1.2	+	-
		80	10±1.5	9±1.1	3±0.7	7±0.8	10±1.5	+	+
		120	9±0.5	7±1.2	2±0.8	5±0.9	9±0.5	+	-
	Metalaxyl	1500	12±1.0	18±1.2	5±0.5	18±1.4	12±1.0	+	+
		3000	11±1.0	14±1.2	4±0.6	11±0.8	11±1.0	+	-
		4500	10±1.0	11±1.2	3±0.7	9±0.3	10±1.0	+	-
	Kitazin	96	12±1.5	19±2.1	6±0.3	18±1.4	12±1.5	+	-
		192	12±1.0	14±1.5	4±0.7	12±0.7	12±1.0	+	+
		288	11±1.0	12±1.4	2±0.5	8±0.5	11±1.0	+	-
Control (without pesticide)			12±1.5	28±2.2	9±0.4	32±1.5	16±1.4	+	+

Table 31 Plant growth promoting activities of phosphate solubilizing bacterium *Pseudomonas putida* strain PS9 in the presence of varying concentrations of herbicides, insecticides and fungicides

Pesticides		Dose rate (µg /l)	Plant growth promoting activities					Ammonia	HCN
			Siderophores			IAA (µg /ml)	EPS (µg /ml)		
			Zone on CAS agar (mm)	SA (µg /ml)	DHBA (µg /ml)	100T			
Herbicides	Quizalafop-p-ethyl	40	10±1.2	38±2.1	11±1.2	13±1.1	19±1.5	+	+
		80	9±1.4	34±1.2	8±0.5	5±0.5	21±1.3	+	+
		120	8±1.0	22±1.3	2±0.6	4±0.6	24±2.7	+	+
	Clodinafop	400	11±1.0	35±1.6	11±0.5	19±1.2	19±1.6	+	+
		800	10±1.0	31±2.7	3±0.5	12±0.8	22±1.2	+	+
		1200	10±1.0	31±1.5	2±0.4	10±0.6	25±2.6	+	+
	Metribuzin	850	11±1.5	27±1.4	9±0.2	25±1.4	18±1.2	+	+
		1700	11±1.1	24±1.5	8±0.5	17±1.2	20±1.1	+	+
		2550	10±1.2	19±1.6	6±0.2	15±1.1	22±1.2	+	+
	Glyphosate	1444	11±1.0	26±1.2	10±0.3	22±1.2	18±1.4	+	+
		2888	11±1.5	18±1.2	6±0.2	13±1.3	21±1.5	+	+
		4332	10±1.0	15±1.3	4±0.2	9±0.5	25±1.5	+	+
Insecticides	Fipronil	200	11±1.0	36±1.4	15±1.2	26±1.2	21±1.7	+	+
		400	10±1.0	33±2.1	7±0.2	15±1.4	23±1.6	+	+
		600	9±1.0	28±1.3	4±0.3	9±1.2	25±1.3	+	+
	Pyriproxyfen	1300	10±1.5	34±1.2	11±0.7	24±2.0	20±1.2	+	+
		2600	9±1.0	31±1.4	4±0.8	14±1.4	21±1.2	+	+
		3900	9±1.0	25±1.3	3±0.6	7±0.6	23±1.2	+	+
	Imidacloprid	100	11±1.5	22±1.2	7±0.5	16±2.1	20±1.6	+	+
		200	11±1.8	17±1.2	4±0.4	7±0.8	20±1.5	+	+
		300	10±1.5	15±1.1	3±0.6	4±0.6	22±1.7	+	+
	Thiamethoxam	25	11±1.0	21±1.1	8±0.8	13±1.5	20±1.4	+	+
		50	10±1.0	16±1.2	5±0.6	9±0.6	22±1.3	+	+
		75	9±1.0	13±1.5	3±0.5	5±0.8	23±2.2	+	+
Fungicides	Tebuconazole	100	10±1.0	22±2.5	8±0.4	8±0.5	20±1.8	+	+
		200	9±1.0	15±1.8	5±0.2	4±0.3	21±1.4	+	+
		300	8±1.0	13±1.7	4±0.2	2±0.7	22±1.5	+	+
	Hexaconazole	40	10±1.0	24±1.6	11±0.3	14±1.5	19±1.9	+	+
		80	9±1.0	19±1.5	7±0.4	7±0.8	20±1.5	+	+
		120	9±1.0	16±1.2	5±0.2	5±0.7	24±1.8	+	+
	Metalaxyl	1500	11±1.0	33±2.1	8±0.4	19±1.8	18±1.8	+	+
		3000	11±1.5	28±1.3	7±0.5	17±1.5	20±1.4	+	+
		4500	10±1.0	23±1.3	5±0.2	14±1.6	22±16	+	+
	Kitazin	96	11±1.5	26±1.4	9±0.3	23±1.4	18±1.5	+	+
		192	11±1.0	19±1.2	6±0.2	16±1.3	19±1.9	+	+
		288	10±1.4	16±1.5	4±0.2	11±1.5	21±1.4	+	+
Control (without pesticide)			11±1.0	41±1.5	17±1.4	34±1.2	17±1.1	+	+

Table 32 Plant growth promoting activities of phosphate solubilizing bacterium *Klebsiella sp.* strain PS19 in the presence of varying concentrations of pesticides

Pesticides		Dose rate (µg /l)	Plant growth promoting activities					Ammonia	HCN
			Siderophores			IAA (µg /ml)	EPS (µg /ml)		
			Zone on CAS agar (mm)	SA (µg /ml)	DHBA (µg /ml)	100T			
Herbicides	Quizalafop-p-ethyl	40	11±1.0	33±2.1	6±0.3	15±1.3	18±1.4	+	+
		80	10±1.0	30±2.3	1±0.5	9±1.2	19±1.3	+	+
		120	9±1.0	25±1.2	1±0.7	7±0.6	21±1.5	+	+
	Clodinafop	400	12±1.5	37±2.1	9±0.5	20±1.5	19±1.6	+	+
		800	11±1.0	30±2.5	5±0.4	14±1.3	21±1.7	+	+
		1200	10±1.0	27±1.6	3±0.4	12±1.2	24±1.3	+	+
	Metribuzin	850	13±1.0	37±2.1	8±0.5	22±1.2	18±1.5	+	+
		1700	12±1.0	31±1.8	7±0.5	13±1.1	20±1.5	+	+
		2550	11±1.5	29±2.1	5±0.3	8±0.6	23±2.0	+	+
	Glyphosate	1444	13±1.0	35±2.5	6±0.6	26±2.8	20±1.2	+	+
		2888	13±1.0	28±1.5	4±0.7	16±1.6	22±1.5	+	+
		4332	12±1.0	25±1.7	3±0.6	8±0.5	25±2.2	+	+
Insecticides	Fipronil	200	12±1.5	42±2.2	6±0.2	33±3.2	19±1.3	+	+
		400	11±1.0	37±2.6	4±0.5	21±2.2	20±1.2	+	+
		600	10±1.0	35±2.2	3±0.3	14±1.4	22±1.2	+	+
	Pyriproxyfen	1300	11±1.0	29±2.4	5±0.7	22±1.5	21±1.5	+	+
		2600	10±1.0	26±1.8	3±0.8	13±1.2	23±1.5	+	+
		3900	9±1.0	23±1.9	2±0.6	9±0.8	24±2.2	+	+
	Imidacloprid	100	13±1.6	32±1.5	4±0.5	20±1.4	19±1.5	+	+
		200	12±1.0	25±2.1	3±0.4	13±1.3	22±1.5	+	+
		300	11±1.0	23±1.7	2±0.6	10±1.2	24±2.2	+	+
	Thiamethoxam	25	13±1.0	34±3.0	6±0.7	19±1.2	19±1.3	+	+
		50	12±1.0	27±2.4	3±0.4	11±1.5	20±1.5	+	+
		75	12±1.5	25±2.5	2±0.4	6±0.3	21±1.3	+	+
Fungicides	Tebuconazole	100	11±1.0	29±1.6	6±0.3	12±1.4	22±1.5	+	+
		200	10±1.2	25±1.7	5±0.2	7±0.6	24±1.4	+	+
		300	9±1.0	22±1.4	3±0.6	3±0.4	28±2.5	+	+
	Hexaconazole	40	11±1.5	30±2.1	4±0.7	18±1.5	21±1.7	+	+
		80	11±1.0	22±1.4	3±0.8	13±0.4	24±2.5	+	+
		120	10±1.3	18±1.6	2±0.9	8±0.5	29±1.2	+	+
	Metalaxyl	1500	12±1.0	35±2.2	7±0.4	25±2.1	21±1.3	+	+
		3000	11±1.0	29±1.5	4±0.3	16±1.4	23±1.9	+	+
		4500	10±1.0	26±1.6	3±0.5	11±1.2	25±2.4	+	+
	Kitazin	96	13±1.5	37±2.5	7±0.5	27±2.0	18±1.5	+	+
		192	12±1.0	31±1.6	4±0.4	15±1.2	19±1.9	+	+
		288	11±1.0	26±1.8	3±0.2	9±0.6	21±1.6	+	+
Control (without pesticide)			13±1.0	47±2.3	10±0.4	42±3.4	18±1.4	+	+

Table 33 Tri-calcium phosphate solubilizing activity of phosphate solubilizing *Pseudomonas aeruginosa* strain PS1 grown in Pikovskaya medium amended with varying concentrations of herbicides, insecticides and fungicides after 7 days of incubation

Pesticides		Dose rate (µg /l)	Phosphate solubilized		
			Liquid medium (µg/ml)	Change in pH	Solubilization index*
Herbicides	Quizalafop-p-ethyl	40	67±4	6.3	1.5
		80	20±2	6.7	1.2
		120	15±3	6.8	1.0
	Clodinafop	400	255±9	5.9	1.8
		800	105±5	6.2	1.5
		1200	59±5	6.5	1.5
	Metribuzin	850	258±3	5.5	2.0
		1700	197±6	6.2	2.0
		2550	121±4	6.4	1.8
	Glyphosate	1444	188±7	5.5	2.0
		2888	112±8	6.4	2.0
		4332	87±5	6.7	1.8
Insecticides	Fipronil	200	220±10	5.6	1.8
		400	97±4	6.8	1.5
		600	75±6	6.8	1.5
	Pyriproxyfen	1300	227±9	5.8	1.5
		2600	122±5	6.1	1.5
		3900	65±3	6.4	1.3
	Imidacloprid	100	108±7	5.6	2.0
		200	77±3	6.2	1.8
		300	45±4	6.5	1.5
	Thiamethoxam	25	126±8	5.8	2.0
		50	77±6	6.4	1.8
		75	42±4	6.5	1.5
Fungicides	Tebuconazole	100	93±5	6.3	1.5
		200	25±2	6.7	1.3
		300	17±3	6.8	1.3
	Hexaconazole	40	87±4	6.4	1.8
		80	42±5	6.6	1.8
		120	19±3	6.8	1.5
	Metalaxyl	1500	107±7	5.7	2.0
		3000	84±6	6.6	1.8
		4500	57±2	6.8	1.8
	Kitazin	96	210±6	5.3	2.0
		192	117±	6.1	1.8
		288	93±	6.5	1.8
Control (without pesticides)			345±8	4.5	2.0

*In this and succeeding tables, solubilization index = (zone diameter-colony diameter)/colony diameter. Values are mean of three replicates

Table 34 Tri-calcium phosphate solubilizing activity of phosphate solubilizing *Enterobacter asburiae* strain PS2 grown in Pikovskaya medium amended with varying concentrations of herbicides, insecticides and fungicides after 7 days of incubation

Pesticides		Dose rate (µg /l)	Phosphate solubilized		
			Liquid medium (µg/ml)	Change in pH	Solubilization index
Herbicides	Quizalafop-p-ethyl	40	71±3	6.5	1.5
		80	23±2	6.8	1.3
		120	15±2	6.8	0.8
	Clodinafop	400	198±7	5.1	2.0
		800	97±5	6.3	1.8
		1200	55±4	6.5	1.3
	Metribuzin	850	212±7	5.6	2.0
		1700	195±8	6.1	2.0
		2550	128±5	6.5	1.8
	Glyphosate	1444	184±4	5.4	2.0
		2888	112±3	5.7	1.8
		4332	67±6	6.2	1.5
Insecticides	Fipronil	200	170±5	5.3	1.8
		400	87±5	6.1	1.8
		600	59±4	6.5	1.3
	Pyriproxyfen	1300	186±6	5.2	1.5
		2600	110±7	6.4	1.3
		3900	36±5	6.5	1.0
	Imidacloprid	100	112±4	5.9	2.0
		200	71±3	6.4	1.5
		300	37±5	6.5	1.5
	Thiamethoxam	25	117±6	5.6	2.0
		50	78±4	6.1	1.8
		75	39±8	6.5	1.8
Fungicides	Tebuconazole	100	86±5	6.4	1.5
		200	29±4	6.7	1.3
		300	17±2	6.8	0.8
	Hexaconazole	40	80±6	6.4	1.8
		80	39±5	6.7	1.8
		120	24±2	6.7	1.3
	Metalaxyl	1500	168±8	5.6	1.8
		3000	127±5	5.8	1.5
		4500	98±3	6.2	1.3
	Kitazin	96	207±8	5.1	2.0
		192	152±9	6.3	1.8
		288	112±6	6.5	1.5
Control (without pesticides)			258±3	4.7	2.0

Table 35 Tri-calcium phosphate solubilizing activity of phosphate solubilizing *Pseudomonas putida* strain PS9 grown in Pikovskaya medium amended with varying concentrations of herbicides, insecticides and fungicides after 7 days of incubation

Pesticides		Dose rate (µg /l)	Phosphate solubilized		
			Liquid medium (µg/ml)	Change in pH	Solubilization index
Herbicides	Quizalafop-p-ethyl	40	33±3	6.5	1.3
		80	25±2	6.8	1.0
		120	17±2	6.8	0.8
	Clodinafop	400	135±6	5.7	1.5
		800	75±5	6.2	1.5
		1200	53±3	6.5	1.3
	Metribuzin	850	201±8	5.7	1.8
		1700	116±6	6.3	1.8
		2550	74±4	6.5	1.5
	Glyphosate	1444	86±5	6.0	1.8
		2888	69±3	6.4	1.5
		4332	41±2	6.7	1.5
Insecticides	Fipronil	200	205±9	5.6	1.5
		400	125±7	5.8	1.3
		600	75±3	6.1	1.3
	Pyriproxyfen	1300	192±8	5.8	1.5
		2600	37±4	6.8	1.3
		3900	22±3	6.8	1.0
	Imidacloprid	100	75±2	6.2	1.8
		200	38±4	6.6	1.5
		300	25±6	6.8	1.5
	Thiamethoxam	25	83±7	6.7	1.8
		50	56±3	6.7	1.8
		75	37±2	6.8	1.8
Fungicides	Tebuconazole	100	97±4	6.5	1.5
		200	26±5	6.8	1.0
		300	17±2	6.8	0.8
	Hexaconazole	40	56±3	6.4	1.5
		80	29±2	6.8	1.3
		120	17±2	6.8	1.0
	Metalaxyl	1500	77±4	5.2	1.8
		3000	42±5	6.1	1.5
		4500	15±3	6.4	1.3
	Kitazin	96	113±7	5.7	1.8
		192	85±4	6.3	1.8
		288	54±3	6.5	1.5
Control (without pesticides)			298±7	4.4	1.8

Table 36 Tri-calcium phosphate solubilizing activity of phosphate solubilizing *Klebsiella sp.* strain PS19 grown in Pikovskaya medium amended with varying concentrations of herbicides, insecticides and fungicides after 7 days of incubation

Pesticides		Dose rate (µg /l)	Phosphate solubilized		
			Liquid medium (µg/ml)	Change in pH	Solubilization index
Herbicides	Quizalafop-p-ethyl	40	22±2	6.7	1.8
		80	15±2	6.8	1.5
		120	9±2	6.8	1.3
	Clodinafop	400	78±4	6.2	2.0
		800	50±5	6.4	1.8
		1200	34±3	6.7	1.5
	Metribuzin	850	192±5	5.8	2.5
		1700	112±7	6.4	2.5
		2550	84±4	6.6	2.3
	Glyphosate	1444	107±5	6.4	2.5
		2888	74±3	6.5	2.3
		4332	47±2	6.5	2.3
Insecticides	Fipronil	200	197±8	5.1	2.0
		400	107±5	5.6	2.0
		600	44±2	5.8	1.8
	Pyriproxyfen	1300	230±8	5.2	2.0
		2600	54±4	6.5	1.8
		3900	14±3	6.8	1.5
	Imidacloprid	100	78±2	6.2	2.3
		200	33±2	6.8	2.3
		300	15±2	6.8	2.0
	Thiamethoxam	25	81±4	6.0	2.5
		50	30±2	6.7	2.3
		75	17±2	6.8	2.3
Fungicides	Tebuconazole	100	50±5	6.7	1.8
		200	29±5	6.8	1.5
		300	17±3	6.8	1.0
	Hexaconazole	40	73±6	6.1	2.0
		80	39±4	6.8	1.8
		120	26±2	6.8	1.5
	Metalaxyl	1500	83±5	6.2	2.3
		3000	52±6	6.4	2.3
		4500	33±3	6.7	1.8
	Kitazin	96	121±9	5.7	2.5
		192	83±6	6.1	2.3
		288	51±5	6.5	2.0
Control (without pesticides)			294±4	4.6	2.5

Table 37 Effect of three concentrations of quizalafop-p-ethyl on growth and symbiotic properties of chickpea plants grown in soil inoculated with *Mesorhizobium* sp. strain MRC4[†] and without bioinoculant

Treatment	Dose rate (µg/ kg soil)	Length/ plant (cm)				Dry biomass (g/ plant)				Nodulation				Total dry biomass (g /plant)	
		Root		Shoot		Root		Shoot		No./ plant		Nodule biomass (mg/ plant)			
		90 DAS	135 DAS	90 DAS	135 DAS	90 DAS	135 DAS	90 DAS	135 DAS	90 DAS	135 DAS	90 DAS	135 DAS	90 DAS	135 DAS
Uninoculated	Control	31	33	34	37	0.88	0.91	2.90	3.80	21	14	180	275	3.96	4.99
	40	15	17	21	22	0.52	0.64	0.86	1.36	18	13	83	107	1.46	2.12
	80	11	13	19	21	0.40	0.45	0.70	0.96	14	10	51	78	1.15	1.49
	120	7	9	16	17	0.20	0.31	0.56	0.51	7	5	25	58	0.79	0.88
Inoculated	Control	33	35	41	42	1.04	1.13	3.63	4.20	38	31	325	297	5.00	5.63
	40	19	20	23	25	0.59	0.67	1.86	2.06	21	17	130	117	2.58	2.85
	80	15	17	21	23	0.44	0.51	1.06	1.14	19	15	103	87	1.60	1.74
	120	10	11	18	20	0.23	0.35	0.73	0.93	17	13	74	66	1.03	1.35
LSD		1.2	1.14	0.84	1.4	0.28	0.15	0.06	0.17	0.22	0.06	0.45	0.56	1.42	1.7
F value	Inoculation (df=1)	128.5*	142.0*	1737*	956*	1.41	451.2*	213.5*	144*	773*	1148*	30.9*	2035*	107.5*	2249*
	Herbicide (df=3)	22.5*	106.5*	327.2*	41.1*	18.2*	202.6*	88.5*	56.1*	123.4*	67.5*	3.5	142.5*	2.2	354.2*
	Inoculation × herbicide (df=3)	2.2	5.4	74.5*	69.5*	45.5*	76.1*	32.4*	3.0	45.1*	153.2*	1.7	75.3*	1.3	139*

In this and succeeding tables, values are mean of three replicates where each replicate constituted three plants/ pot. [†]Strain MRC4 at 0, 40, 80 and 120 µg L^{-1} of quizalafop-p-ethyl, produced 35, 25, 22 and 19 µg L^{-1} SA; 19, 15, 13 and 10 µg L^{-1} DHBA, 44, 19, 15 and 11 µg ml^{-1} IAA and 21, 21, 21 and 23 µg ml^{-1} EPS, respectively. *Significantly different from the control at $p \leq 0.05$.

Table 38 Effect of three concentrations of clodinafop on growth and symbiotic properties of chickpea plants grown in soil inoculated with *Mesorhizobium* sp. strain MRC4[†] and without bioinoculant

Treatment	Dose rate (µg/ kg soil)	Length/ plant (cm)				Dry biomass (g/ plant)				Nodulation				Total dry biomass (g /plant)	
		Root		Shoot		Root		Shoot		No./ plant		Nodule biomass (mg/ plant)			
		90 DAS	135 DAS	90 DAS	135 DAS	90 DAS	135 DAS	90 DAS	135 DAS	90 DAS	135 DAS	90 DAS	135 DAS	90 DAS	135 DAS
Uninoculated	Control	31	33	34	37	0.88	0.91	2.90	3.80	21	14	180	275	3.96	4.99
	400	27	29	32	35	0.80	0.87	2.63	2.83	20	12	160	210	3.59	3.91
	800	21	23	29	32	0.69	0.78	2.26	3.26	19	10	146	176	3.10	4.22
	1200	18	20	27	27	0.62	0.69	1.80	2.10	16	9	133	124	2.55	2.92
Inoculated	Control	33	35	41	42	1.04	1.13	3.63	4.20	38	31	325	297	5.00	5.63
	400	30	32	38	40	0.83	0.92	3.16	3.90	36	26	260	224	4.25	5.04
	800	27	28	33	35	0.73	0.85	2.80	3.56	28	22	229	190	3.76	4.60
	1200	21	25	31	33	0.64	0.75	2.40	2.73	26	21	157	136	3.20	3.62
LSD		2.1	1.5	1.7	0.7	1.5	0.06	1.3	0.15	1.3	0.04	0.11	0.13	3.03	2.1
F value	Inoculation (df=1)	327.6*	43.2*	898.4*	26.5*	1252*	46.1*	1223*	737*	1335.7*	21.0*	147*	44.0*	325.6*	45.7*
	Herbicide (df=3)	15.2*	1.4	124.7*	5.2*	103.3*	11.8*	134.2*	63.5*	31.4*	0.5	2.3	2.2	75.2*	1.2
	Inoculation × herbicide (df=3)	25.9*	2.3	61.9*	18.6*	78.3*	1.7	78.3*	403.6*	129.9*	0.4	17.5*	2.8	22.9*	1.7

[†]Strain MRC4 at 400, 800 and 1200 µg L^{-1} of clodinafop, produced 32, 30 and 27 µg L^{-1} SA; 17, 15 and 12 µg L^{-1} DHBA; 33, 23 and 18 µg ml^{-1} IAA and 22, 25 and 27 µg ml^{-1} EPS, respectively

Table 39 Effect of three concentrations of fipronil on growth and symbiotic properties of chickpea plants grown in soil inoculated with *Mesorhizobium* sp. strain MRC4[†] and without bioinoculant

Treatment	Dose rate (µg/ kg soil)	Length/ plant (cm)				Dry biomass (g/ plant)				Nodulation				Total dry biomass (g /plant)	
		Root		Shoot		Root		Shoot		No./ plant		Nodule biomass (mg/ plant)			
		90 DAS	135 DAS	90 DAS	135 DAS	90 DAS	135 DAS	90 DAS	135 DAS	90 DAS	135 DAS	90 DAS	135 DAS	90 DAS	135 DAS
Uninoculated	Control	31	33	34	37	0.88	0.91	2.90	3.80	21	14	180	275	3.96	4.99
	200	25	26	29	31	0.55	0.75	1.73	2.36	17	9	152	139	2.44	3.25
	400	17	20	25	27	0.49	0.68	1.16	1.93	17	7	129	114	1.78	2.72
	600	14	16	24	26	0.42	0.55	1.01	1.63	14	4	109	100	1.54	2.28
Inoculated	Control	33	35	41	42	1.04	1.13	3.63	4.20	38	31	325	297	5.00	5.63
	200	28	30	30	31	0.62	0.77	1.86	2.80	34	26	296	291	2.78	3.86
	400	22	24	27	28	0.55	0.73	1.52	2.30	26	18	243	287	2.31	3.32
	600	19	21	25	23	0.47	0.62	1.30	1.90	22	18	226	278	2.00	2.80
LSD		1.5	1.1	0.75	1.2	3.6	4.3	2.2	2.7	1.4	0.91	1.5	1.8	4.5	5.2
F value	Inoculation (df=1)	154*	435.2*	1194*	782*	176.4*	612.2*	288.4*	151.3*	1084*	227*	465*	715.3*	432*	337.2*
	Insecticide (df=3)	55.4*	138.7*	254.3*	130.4*	60.5*	104.5*	29*	84.5*	335.3*	71.2*	179*	213.2*	120.4*	125.4*
	Inoculation × insecticide (df=3)	12.8*	65.2*	44.2*	122.2*	7.4	58.3*	12.5*	32.9*	95.6*	27.1*	72.4*	42.1*	28.5*	1.4

[†]Strain MRC4 at 200, 400 and 600 µg L^{-1} of fipronil, produced 30, 28 and 27 µg L^{-1} SA; 18, 15 and 13 µg L^{-1} DHBA; 33, 27 and 22 µg ml^{-1} IAA and 22, 24 and 26 µg ml^{-1} EPS, respectively

Table 40 Effect of three concentrations of pyriproxyfen on growth and symbiotic properties of chickpea plants grown in soil inoculated with *Mesorhizobium* sp. strain MRC4[†] and without bioinoculant

Treatment	Dose rate (µg/ kg soil)	Length/ plant (cm)				Dry biomass (g/ plant)				Nodulation				Total dry biomass (g /plant)	
		Root		Shoot		Root		Shoot		No./ plant		Nodule biomass (mg/ plant)			
		90 DAS	135 DAS	90 DAS	135 DAS	90 DAS	135 DAS	90 DAS	135 DAS	90 DAS	135 DAS	90 DAS	135 DAS	90 DAS	135 DAS
Uninoculated	Control	31	33	34	37	0.88	0.91	2.90	3.80	21	14	180	275	3.96	4.99
	1300	21	23	29	31	0.67	0.49	1.33	1.93	20	8	136	132	2.14	2.55
	2600	17	19	24	27	0.56	0.43	0.96	1.40	19	6	119	99	1.64	1.93
	3900	14	16	19	21	0.47	0.28	0.81	1.30	18	6	106	71	1.39	1.68
Inoculated	Control	33	35	41	42	1.04	1.13	3.63	4.20	38	31	325	297	5.00	5.63
	1300	25	26	31	33	0.54	0.78	1.53	2.66	28	21	312	288	2.38	3.73
	2600	20	22	26	28	0.46	0.68	1.23	1.70	25	20	285	280	1.98	2.66
	3900	17	19	21	23	0.37	0.58	0.96	1.35	21	17	217	276	1.55	2.21
LSD		1.6	1.3	0.76	1.2	3.6	4.7	3.6	2.2	0.5	0.72	0.75	1.1	3.8	4.6
F value	Inoculation (df=1)	523*	329*	929.5*	331*	388.5*	222*	519*	631*	306.4*	417*	231.1*	873.2*	237*	227.3*
	Insecticide (df=3)	109.2*	112*	234.5*	84.1*	42.1*	73.2*	203.4*	128.4*	72.1*	122.3*	102.4*	271*	107.3*	58.2*
	Inoculation × insecticide (df=3)	18.2*	23.5*	43*	16.5*	65.2*	19*	63.5*	73.1*	17.2*	56.1*	48*	78.3*	23.7*	18.5*

[†]Strain MRC4 at 1300, 2600 and 3900 µg L^{-1} of pyriproxyfen, produced 28, 25 and 21 µg L^{-1} SA; 16, 15 and 12 µg L^{-1} DHBA; 29, 21 and 17 µg ml^{-1} IAA and 23, 24 and 26 µg ml^{-1} EPS, respectively

Table 41 Effect of three concentrations of tebuconazole on growth and symbiotic properties of chickpea plants grown in soil inoculated with *Mesorhizobium* sp. strain MRC4[†] and without bioinoculant

Treatment	Dose rate (µg/ kg soil)	Length/ plant (cm)				Dry biomass (g/ plant)				Nodulation				Total dry biomass (g /plant)	
		Root		Shoot		Root		Shoot		No./ plant		Nodule biomass (mg/ plant)			
		90 DAS	135 DAS	90 DAS	135 DAS	90 DAS	135 DAS	90 DAS	135 DAS	90 DAS	135 DAS	90 DAS	135 DAS	90 DAS	135 DAS
Uninoculated	Control	31	33	34	37	0.88	0.91	2.90	3.80	21	14	180	275	3.96	4.99
	100	21	23	24	26	0.46	0.65	1.10	1.80	16	14	116	124	1.68	2.57
	200	15	17	19	21	0.39	0.54	0.91	1.33	14	13	97	97	1.40	1.97
	300	11	12	17	19	0.34	0.42	0.66	1.10	11	9	80	75	1.08	1.70
Inoculated	Control	33	35	41	42	1.04	1.13	3.63	4.20	38	31	325	297	5.00	5.63
	100	25	28	26	29	0.49	0.74	1.40	2.33	28	23	151	135	2.04	3.21
	200	18	21	21	26	0.44	0.69	1.03	1.83	25	17	121	111	1.59	2.63
	300	13	16	19	23	0.38	0.47	0.76	1.26	20	16	96	85	1.24	1.82
LSD		2.5	2.4	1.5	1.9	2.6	2.8	7.2	6.8	1.2	0.8	1.5	2.4	6.7	8.7
F value	Inoculation (df=1)	521*	1047*	741*	735*	1161*	1311*	656*	1125*	2547*	1165*	4288*	654*	448*	346.5*
	Fungicide (df=3)	104.5*	432*	66.4*	41.5*	359*	134*	57.5*	327*	562*	127.2*	443*	119.4*	56.3*	62.4*
	Inoculation × fungicide (df=3)	32.5*	240*	21.3*	561.6*	404*	458.5*	28.4*	269*	459*	543.7*	137*	32.5*	14.5*	21.2*

[†]Strain MRC4 at 100, 200 and 300 µg L^{-1} of tebuconazole, produced 26, 23 and 21 µg L^{-1} SA; 13, 10 and 8 µg L^{-1} DHBA; 17, 14 and 11 µg ml^{-1} IAA and 22, 23 and 25 µg ml^{-1} EPS, respectively

Table 42 Effect of three concentrations of quizalafop-p-ethyl on biological and chemical properties of chickpea plants grown in soil inoculated with *Mesorhizobium* sp. strain MRC4 and without bioinoculant

Treatment	Dose rate (µg/ kg soil)	Leghaemoglobin content [mM (g f.m.)$^{-1}$]	Chlorophyll content (mg/g)	N content (mg/g)		P content (mg/g)		Seed yield (g/plant)	Seed protein (mg/g)
				Root	Shoot	Root	Shoot		
Uninoculated	Control	0.13	1.96	18	27	0.17	0.21	2.7	241
	40	0.03	1.45	14	20	0.13	0.17	1.2	185
	80	0.02	1.38	12	19	0.11	0.15	0.8	176
	120	0.01	1.31	10	17	0.10	0.13	0.6	163
Inoculated	Control	0.19	2.43	24	32	0.23	0.27	3.7	260
	40	0.04	2.31	17	24	0.16	0.23	1.7	235
	80	0.03	1.94	16	23	0.13	0.19	1.2	229
	120	0.01	1.91	13	20	0.12	0.17	0.9	223
LSD		0.003	0.05	1.4	1.6	0.004	0.002	0.03	5.6
F value	Inoculation (df= 1)	434.5*	705.6*	201.4*	547.5*	201.4*	304.3*	1157*	1042*
	Herbicides (df=3)	83.2*	97.3*	24.3*	64.2*	91.6*	55.2*	187.3*	305*
	Inoculation × herbicide (df=3)	21.4*	33.4*	19.3*	62.3*	10.9*	31.6*	495.5*	120.5*

Table 43 Effect of three concentrations of clodinafop on biological and chemical properties of chickpea plants grown in soil inoculated with *Mesorhizobium* sp. strain MRC4 and without bioinoculant

Treatment	Dose rate (µg/ kg soil)	Leghaemoglobin content [mM (g f.m.)$^{-1}$]	Chlorophyll content (mg/g)	N content (mg/g)		P content (mg/g)		Seed yield (g/plant)	Seed protein (mg/g)
				Root	Shoot	Root	Shoot		
Uninoculated	Control	0.13	1.96	18	27	0.17	0.21	2.7	241
	400	0.06	1.86	17	26	0.16	0.19	2.4	235
	800	0.04	1.78	15	24	0.14	0.18	2.2	231
	1200	0.01	1.72	14	23	0.11	0.15	1.7	225
Inoculated	Control	0.19	2.43	24	32	0.23	0.27	3.7	260
	400	0.17	2.41	21	29	0.20	0.25	2.8	258
	800	0.15	2.25	18	27	0.19	0.23	2.4	252
	1200	0.13	2.17	16	24	0.17	0.20	2.1	247
LSD		0.002	0.11	0.41	0.62	0.005	0.008	0.13	6.2
F value	Inoculation (df= 1)	584*	205.4*	325*	924.4*	172.5*	305.3*	870.7*	1055*
	Herbicide (df=3)	25.2*	37.5*	35.5*	95.3*	64.2*	82.6	182.3*	508.2*
	Inoculation × herbicide df=3)	81.5*	14.6*	83.7*	135.6*	16.1*	12.4*	78.5*	148.5*

Table 44 Effect of three concentrations of fipronil on biological and chemical properties of chickpea plants grown in soil inoculated with *Mesorhizobium* sp. strain MRC4 and without bioinoculant

Treatment	Dose rate (µg/ kg soil)	Leghaemoglobin content [mM (g f.m.)$^{-1}$]	Chlorophyll content (mg/g)	N content (mg/g)		P content (mg/g)		Seed yield (g/plant)	Seed protein (mg/g)
				Root	Shoot	Root	Shoot		
Uninoculated	Control	0.13	1.96	18	27	0.17	0.21	2.7	241
	200	0.06	1.79	17	25	0.16	0.19	2.1	230
	400	0.04	1.71	14	24	0.13	0.17	1.7	227
	600	0.03	1.65	13	23	0.12	0.16	1.3	221
Inoculated	Control	0.19	2.43	24	32	0.23	0.27	3.7	260
	200	0.16	2.38	22	30	0.22	0.25	3.5	256
	400	0.13	2.25	21	28	0.20	0.23	3.2	250
	600	0.10	2.17	19	28	0.19	0.22	2.9	246
LSD		0.006	0.04	1.6	2.2	0.004	0.005	0.27	2.2
F value	Inoculation (df= 1)	374.1*	386.5*	186.4*	518.4*	428.9*	238.4*	325.5*	487.5*
	Insecticide (df=3)	18.5*	82.4*	32.1*	114*	104.5*	101.2*	61.1*	1.6
	Inoculation × insecticide (df=3)	1.5	22.6*	17.2*	21.7*	27.8*	24.1*	21.4*	87.5*

Table 45 Effect of three concentrations of pyriproxyfen on biological and chemical properties of chickpea plants grown in soil inoculated with *Mesorhizobium* sp. strain MRC4 and without bioinoculant

Treatment	Dose rate (µg/ kg soil)	Leghaemoglobin content [mM (g f.m.)$^{-1}$]	Chlorophyll content (mg/g)	N content (mg/g)		P content (mg/g)		Seed yield (g/plant)	Seed protein (mg/g)
				Root	Shoot	Root	Shoot		
Uninoculated	Control	0.13	1.96	18	27	0.17	0.21	2.7	241
	1300	0.04	1.68	15	24	0.17	0.20	1.8	228
	2600	0.03	1.61	14	22	0.12	0.18	1.5	222
	3900	0.02	1.56	13	20	0.10	0.15	1.1	198
Inoculated	Control	0.19	2.43	24	32	0.23	0.27	3.7	260
	1300	0.14	2.28	24	31	0.23	0.24	3.4	251
	2600	0.12	2.18	20	29	0.21	0.22	3.0	245
	3900	0.07	2.06	18	28	0.19	0.21	2.8	242
LSD		0.03	0.07	2.1	1.4	0.005	0.002	0.02	3.6
F value	Inoculation (df= 1)	1424*	417.4*	263.5*	345.6*	243.2*	432.5*	2104*	718.4*
	Insecticide (df=3)	255.2*	104.2*	27.5*	32.1*	29.3*	34.7*	319.2*	5.4*
	Inoculation × insecticide (df=3)	64.5*	29.1*	12.2*	13.5*	12.3*	14.2*	64.2*	104.6*

Table 46 Effect of three concentrations of tebuconazole on biological and chemical properties of chickpea plants grown in soil inoculated with *Mesorhizobium* sp. strain MRC4 and without bioinoculant

Treatment	Dose rate (µg/ kg soil)	Leghaemoglobin content [mM (g f.m.)$^{-1}$]	Chlorophyll content (mg/g)	N content (mg/g)		P content (mg/g)		Seed yield (g/plant)	Seed protein (mg/g)
				Root	Shoot	Root	Shoot		
Uninoculated	Control	0.13	1.96	18	27	0.17	0.21	2.7	241
	100	0.04	1.62	15	21	0.15	0.17	1.7	209
	200	0.03	1.55	13	20	0.13	0.15	1.5	196
	300	0.01	1.43	12	17	0.12	0.14	0.9	184
Inoculated	Control	0.19	2.43	24	32	0.23	0.27	3.7	260
	100	0.05	2.19	18	26	0.19	0.24	2.3	237
	200	0.03	2.15	16	24	0.17	0.22	1.9	232
	300	0.02	1.96	13	22	0.14	0.21	1.2	228
LSD		0.009	0.14	1.4	1.6	0.006	0.008	0.41	2.4
F value	Inoculation (df= 1)	152.3*	247*	540.3*	142.3*	152*	2475*	272.4*	424*
	Fungicide (df=3)	36.4*	81*	85.4*	32.2*	38.2*	27.2*	45.6*	203.9*
	Inoculation × fungicide (df=3)	6.2*	12.1*	52.3*	7.4*	9.2*	90.2*	15.1*	38.8*

Table 47 Effect of three concentrations of quizalafop-p-ethyl on growth and nodulation of pea plants grown in soil inoculated with and without *Rhizobium* sp. strain MRP1[†]

Treatment	Dose rate (µg/ kg soil)	Length/ plant (cm)				Dry biomass (g/ plant)				Nodulation				Total dry biomass (g /plant)	
		Root		Shoot		Root		Shoot		No./ plant		Dry biomass (mg/ plant)			
		90 DAS	120 DAS	90 DAS	120 DAS	90 DAS	120 DAS	90 DAS	120 DAS	90 DAS	120 DAS	90 DAS	120 DAS	90 DAS	120 DAS
Uninoculated	Control	20	27	24	36	0.35	0.92	1.36	2.07	27	15	283	65	1.99	3.06
	40	13	10	17	20	0.18	0.62	0.66	1.04	7	-	176	-	1.02	1.66
	80	11	7	15	14	0.15	0.52	0.43	0.76	-	-	-	-	0.58	1.28
	120	9	6	12	14	0.12	0.39	0.33	0.65	-	-	-	-	0.45	1.04
Inoculated	Control	29	33	33	57	0.67	0.73	1.40	2.66	36	19	373	124	2.44	3.51
	40	19	12	19	23	0.45	0.48	0.95	2.14	13	-	190	-	1.59	2.62
	80	18	10	17	18	0.36	0.37	0.86	1.37	6	-	123	-	1.34	1.74
	120	16	10	15	16	0.31	0.29	0.77	1.09	-	-	-	-	1.08	1.38
LSD		1.8	1.3	1.4	1.6	0.09	0.05	0.17	0.19	1.6	1.3	1.8	1.4	1.9	2.2
F value	Inoculation (df= 1)	325.6*	1252*	898*	737*	45.7*	36.1*	16.5*	11.0*	720*	28*	65.8*	793.5*	158*	63.5*
	Herbicide (df=3)	5.2*	13.3*	21.7*	23.5*	1.2	1.8	5.2*	0.4	38.5*	2023*	2.8	7938*	2.7	1.5
	Inoculation × herbicide (df=3)	22.9*	78.3*	61.9*	43.6*	1.8	1.9	18.6*	0.6	83.4*	28*	3.6	2380*	21.3*	2.3

[†]Strain MRP1 at 0, 40, 80 and 120 µg L^{-1} of quizalafop-p-ethyl, produced 32, 22, 19 and 14 µg L^{-1} SA; 22, 15, 14 and 10 µg L^{-1} DHBA, 32, 23, 21 and 18 µg ml^{-1} IAA and 20, 20, 22 and 23 µg ml^{-1} EPS, respectively

Table 48 Effect of three concentrations of clodinafop on growth and nodulation of pea plants grown in soil inoculated with *Rhizobium* sp. strain MRP1[†] and without bioinoculant

Treatment	Dose rate (µg/ kg soil)	Length/ plant (cm)				Dry biomass (g/ plant)				Nodulation				Total dry biomass (g /plant)	
		Root		Shoot		Root		Shoot		No./ plant		Dry biomass (mg/ plant)			
		90 DAS	120 DAS	90 DAS	120 DAS	90 DAS	120 DAS	90 DAS	120 DAS	90 DAS	120 DAS	90 DAS	120 DAS	90 DAS	120 DAS
Uninoculated	Control	20	27	24	36	0.35	0.92	1.36	2.07	27	15	283	65	1.99	3.06
	400	19	25	20	32	0.32	0.85	1.26	1.73	19	9	253	49	1.83	2.63
	800	18	18	19	26	0.28	0.74	0.86	1.31	16	-	210	-	1.35	2.05
	1200	16	16	17	25	0.24	0.55	0.80	1.08	11	-	170	-	1.21	1.63
Inoculated	Control	29	33	33	57	0.67	0.73	1.40	2.66	36	19	373	124	2.44	3.51
	400	28	29	31	40	0.60	0.68	1.29	2.32	24	18	290	57	2.18	3.06
	800	26	24	29	35	0.51	0.63	1.12	2.12	20	9	236	50	1.87	2.80
	1200	25	19	22	31	0.42	0.50	0.91	1.61	13	-	270	-	1.60	2.11
LSD		1.1	1.3	1.2	1.4	0.13	0.19	0.08	0..24	0.21	0.08	0.67	0.34	1.24	1.54
F value	Inoculation (df=1)	1612*	1112*	2714*	709*	15.3*	141.2*	46.5*	111*	1.5	21.3*	34.8*	163*	1613*	1224*
	Herbicide (df=3)	41.3*	41.4*	71.6*	54.1*	0.5	2.2	9.6*	3.4	14.5*	2 5	2.8	17.2*	43.5*	37.5*
	Inoculation × herbicide (df=3)	72.2*	65.3*	121*	41.6*	0.9	9.3*	2.1	2.7	24.7*	2.3	1.5	6.7	71.4*	61.2*

[†]Strain MRP1 at 400, 800 and 1200 µg L^{-1} of clodinafop, produced 28, 25 and 21 µg L^{-1} SA; 20, 18 and 15 µg L^{-1} DHBA; 30, 28 and 25 µg ml^{-1} IAA and 21, 23 and 26 µg ml^{-1} EPS, respectively

Table 49 Effect of three concentrations of fipronil on growth and nodulation of pea grown in soil inoculated with *Rhizobium* sp. strain MRP1[†] and without bioinoculant

Treatment	Dose rate (µg/ kg soil)	Length/ plant (cm)				Dry biomass (g/ plant)				Nodulation				Total dry biomass (g /plant)	
		Root		Shoot		Root		Shoot		No./ plant		Dry biomass (mg/ plant)			
		90 DAS	120 DAS	90 DAS	120 DAS	90 DAS	120 DAS	90 DAS	120 DAS	90 DAS	120 DAS	90 DAS	120 DAS	90 DAS	120 DAS
Uninoculated	Control	20	27	24	36	0.35	0.92	1.36	2.07	27	15	283	65	1.99	3.06
	200	18	17	17	27	0.29	0.80	0.91	1.84	15	9	236	34	1.44	2.68
	400	16	16	15	24	0.26	0.73	0.80	1.33	14	7	166	26	1.23	2.09
	600	15	15	13	17	0.24	0.57	0.66	0.97	9	-	132	-	1.03	1.54
Inoculated	Control	29	33	33	57	0.67	0.73	0.14	2.66	36	19	373	124	2.44	3.51
	200	26	14	28	32	0.57	0.68	1.20	2.33	20	16	276	59	1.95	3.07
	400	24	12	24	30	0.50	0.54	1.16	2.36	16	11	213	47	1.87	2.95
	600	21	10	23	20	0.40	0.43	0.97	1.49	10	-	140	-	1.51	1.92
LSD		2.1	1.6	3.2	3.5	0.6	0.4	0.43	0.51	2.3	1.4	0.27	0.36	0.47	0.39
F value	Inoculation (df=1)	925*	225*	459*	394*	14.5*	33.1*	2.6	19*	128.1*	468*	32.7*	44*	92.6*	7.9*
	Insecticide (df=3)	57.8*	17*	20.8*	19.8*	1.2	2.9	0.4	2.3	3.0*	16*	5.9*	21.3*	7.6*	1.0
	Inoculation × insecticide (df=3)	62.4*	9.3*	24.7*	23.9*	0.7	1.2	0.1	1.5	6.0*	29*	2.8	4.1*	5.0*	0.4

[†]Strain MRP1 at 200, 400 and 600 µg L^{-1} of fipronil, produced 30, 28 and 25 µg L^{-1} SA; 18, 17 and 15 µg L^{-1} DHBA; 25, 24 and 21 µg ml^{-1} IAA and 21, 25 and 25 µg ml^{-1} EPS, respectively

Table 50 Effect of three concentrations of pyriproxyfen on growth and nodulation of pea plants grown in soil inoculated with *Rhizobium* sp. strain MRP1[†] and without bioinoculant

Treatment	Dose rate (µg/ kg soil)	Length/ plant (cm)				Dry biomass (g/ plant)				Nodulation				Total dry biomass (g /plant)	
		Root		Shoot		Root		Shoot		No./ plant		Dry biomass (mg/ plant)			
		90 DAS	120 DAS	90 DAS	120 DAS	90 DAS	120 DAS	90 DAS	120 DAS	90 DAS	120 DAS	90 DAS	120 DAS	90 DAS	120 DAS
Uninoculated	Control	20	27	24	36	0.35	0.92	1.36	2.07	27	15	283	65	1.99	3.06
	1300	18	17	17	23	0.25	0.83	0.86	1.18	16	9	213	25	1.32	2.24
	2600	15	13	12	17	0.22	0.63	0.70	0.93	15	-	176	-	1.10	1.56
	3900	13	8	20	14	0.19	0.55	0.60	0.81	10	-	34	-	0.82	1.36
Inoculated	Control	29	33	33	57	0.67	0.73	1.40	2.66	36	19	373	124	2.44	3.51
	1300	24	21	26	32	0.57	0.62	1.14	1.87	18	16	206	59	1.92	2.55
	2600	22	16	26	26	0.39	0.57	1.02	1.65	17	12	166	49	1.58	2.27
	3900	20	11	21	19	0.33	0.46	0.91	1.19	12	-	90	-	1.33	1.65
LSD		1.6	1.2	1.9	2.3	0.32	0.21	0.7	0.8	2.2	1.2	0.27	0.7	1.6	2.5
F value	Inoculation (df=1)	418.5*	247.1*	317*	522.6*	527*	2013*	2.1	23.2*	327*	813*	22.3*	72*	15.3*	23.5*
	Insecticide (df=3)	24.2*	24.3*	19.1*	31.7*	42.4*	287*	0.5	2.8	12.2*	31.6*	4.7*	21.6*	1.2	1.0
	Inoculation × insecticide (df=3)	388.3*	8.2*	12.4*	25.8*	21.7*	68.9*	0.2	1.2	21.3*	67.4*	1.5	6.2*	0.83	1.6

[†]Strain MRP1 at 1300, 2600 and 3900 µg L^{-1} of pyriproxyfen, produced 26, 23 and 21 µg L^{-1} SA; 16, 14 and 12 µg L^{-1} DHBA; 29, 26 and 23 µg ml^{-1} IAA and 22, 23 and 26 µg ml^{-1} EPS respectively

Table 51 Effect of three concentrations of tebuconazole on growth and nodulation of pea plants grown in soil inoculated with *Rhizobium* sp. strain MRP1 and without bioinoculant

Treatment	Dose rate (µg/ kg soil)	Length/ plant (cm)				Dry biomass (g/ plant)				Nodulation				Total dry biomass (g /plant)	
		Root		Shoot		Root		Shoot		No./ plant		Dry biomass (mg/ plant)			
		90 DAS	120 DAS	90 DAS	120 DAS	90 DAS	120 DAS	90 DAS	120 DAS	90 DAS	120 DAS	90 DAS	120 DAS	90 DAS	120 DAS
Uninoculated	Control	20	27	24	36	0.35	0.92	1.36	2.07	27	15	283	65	1.99	3.06
	100	17	12	20	18	0.22	0.54	0.63	1.42	9	7	176	29	1.04	2.19
	200	15	9	19	16	0.19	0.43	0.56	1.04	9	-	133	-	0.88	1.60
	300	13	7	17	13	0.16	0.32	0.50	0.72	-	-	-	-	0.66	1.17
Inoculated	Control	29	33	33	57	0.67	0.76	1.40	2.66	36	19	373	124	2.44	3.51
	100	22	16	24	27	0.55	0.64	1.08	2.45	14	12	296	52	1.93	3.14
	200	20	12	18	19	0.36	0.56	0.88	1.99	10	11	196	45	1.34	2.47
	300	18	10	18	17	0.29	0.45	0.80	1.13	-	8	-	36	1.09	1.49
LSD		1.6	1.2	1.7	1.3	1.8	2.2	2.8	3.7	1.2	1.6	1.3	0.6	3.7	4.1
F value	Inoculation (df=1)	429*	1215*	1143*	444.2*	125.6*	127.5*	173.4*	317*	341.7*	766*	327.2*	515*	382.4*	616*
	Fungicides (df=3)	54.1*	66.3*	64.4*	39.5*	42.6*	54.1*	25.4*	72.4*	57.9*	123*	93.2*	127*	134.2*	232*
	Inoculation × fungicide (df=3)	32.3*	128.6*	129.2*	35.3*	21.3*	1.7	65.2*	32.2*	3.7	65*	33.6*	83.2*	61.3*	51*

†Strain MRP1 at 100, 200 and 300 µg L^{-1} of tebuconazole, produced 24, 20 and 18 µg L^{-1} SA; 15, 10 and 9 µg L^{-1} DHBA; 22, 17 and 16 µg ml^{-1} IAA and 22, 24 and 25 µg ml^{-1} EPS, respectively

Table 52 Effect of three concentrations of quizalafop-p-ethyl on chlorophyll, leghaemoglobin, N and P content, seed yield and grain protein of pea plants grown in soil inoculated with *Rhizobium* sp. strain MRP1 and without bioinoculant

Treatment	Dose rate (µg/ kg soil)	Leghaemoglobin content [mM (g f.m.)$^{-1}$]	Chlorophyll content (mg/g)	N content (mg/g)		P content (mg/g)		Seed yield (g/plant)	Seed protein (mg/g)
				Root	Shoot	Root	Shoot		
Uninoculated	Control	0.17	0.75	34	45	0.21	0.28	7.4	224
	40	0.10	0.68	29	37	0.17	0.23	5.3	220
	80	-	0.66	27	35	0.15	0.21	4.8	218
	120	-	0.63	26	29	0.13	0.18	3.7	216
Inoculated	Control	0.23	0.89	40	52	0.30	0.36	9.6	240
	40	0.16	0.74	35	43	0.24	0.29	6.5	232
	80	0.13	0.71	33	39	0.22	0.27	5.7	230
	120	-	0.69	29	34	0.19	0.24	4.5	228
LSD		0.03	0.13	1.1	1.4	0.06	0.07	0.37	1.8
F value	Inoculation (df= 1)	412.2*	54.3*	37*	83.3*	217*	94*	112.6*	2172*
	Herbicide (df=3)	6.3*	2.5	4.4*	7.2*	0	0.6	7.5*	44.6*
	Inoculation × herbicide (df=3)	41.3*	2.1	24.6*	6.3*	13.5*	9.4*	5.3*	91.4*

Table 53 Effect of three concentrations of clodinafop on chlorophyll, leghaemoglobin, N and P content, seed yield and grain protein of pea plants grown in soil inoculated with *Rhizobium* sp. strain MRP1 and without bioinoculant

Treatment	Dose rate (µg/ kg soil)	Leghaemoglobin content [mM (g f.m.)$^{-1}$]	Chlorophyll content (mg/g)	N content (mg/g)		P content (mg/g)		Seed yield (g/plant)	Seed protein (mg/g)
				Root	Shoot	Root	Shoot		
Uninoculated	Control	0.17	0.75	34	45	0.21	0.28	7.4	224
	400	0.16	0.73	34	42	0.19	0.28	7.1	224
	800	0.14	0.71	32	39	0.18	0.26	6.9	223
	1200	0.13	0.69	29	32	0.16	0.23	6.4	221
Inoculated	Control	0.23	0.89	40	52	0.30	0.36	9.6	240
	400	0.22	0.87	39	49	0.29	0.33	9.3	239
	800	0.21	0.85	37	44	0.27	0.31	8.8	238
	1200	0.19	0.83	35	35	0.25	0.28	8.3	237
LSD		0.006	0.07	1.4	0.7	0.008	0.02	0.27	1.3
F value	Inoculation (df=1)	327*	119.3*	619*	238.5*	649.7*	1312*	278.5*	2408*
	Herbicide (df=3)	12.5*	2.1	9.3*	7.6*	8.2*	21.5*	9.4*	44.2*
	Inoculation × herbicide (df=3)	19.5*	8.7*	22.8*	24.8*	15.2*	25.6*	13.6*	71.7*

Table 54 Effect of three concentrations of fipronil on chlorophyll, leghaemoglobin, N and P content, seed yield and grain protein of pea plants grown in soil inoculated with *Rhizobium* sp. strain MRP1 and without bioinoculant

Treatment	Dose rate (µg/ kg soil)	Leghaemoglobin content [mM (g f.m.)$^{-1}$]	Chlorophyll content (mg/g)	N content (mg/g)		P content (mg/g)		Seed yield (g/plant)	Seed protein (mg/g)
				Root	Shoot	Root	Shoot		
Uninoculated	Control	0.17	0.75	34	45	0.21	0.28	7.4	224
	200	0.15	0.74	31	40	0.20	0.27	6.8	222
	400	0.13	0.71	27	38	0.19	0.25	6.5	221
	600	0.11	0.68	25	33	0.15	0.23	6.3	221
Inoculated	Control	0.23	0.89	40	52	0.30	0.36	9.6	240
	200	0.20	0.86	36	50	0.28	0.32	8.7	240
	400	0.18	0.84	34	45	0.25	0.29	7.6	238
	600	0.15	0.81	32	41	0.23	0.27	7.2	236
LSD		0.005	0.09	1.6	1.2	0.008	0.03	2.5	6.7
F value	Inoculation (df=1)	260.6*	13.9*	215.4*	179.7*	645.3*	882.5*	1401*	102*
	Insecticide (df=3)	13.6*	0.6	312.2*	8.5*	28.4*	36.1*	30.4*	3.5*
	Inoculation × insecticide (df=3)	625.1*	1.1	21.5*	12.4*	51.6*	70.5*	129*	9.9*

Table 55 Effect of three concentrations of pyriproxyfen on chlorophyll, leghaemoglobin, N and P content, seed yield and grain protein of pea plants grown in soil inoculated with *Rhizobium* sp. strain MRP1 and without bioinoculant

Treatment	Dose rate (µg/ kg soil)	Leghaemoglobin content [mM (g f.m.)$^{-1}$]	Chlorophyll content (mg/g)	N content (mg/g)		P content (mg/g)		Seed yield (g/plant)	Seed protein (mg/g)
				Root	Shoot	Root	Shoot		
Uninoculated	Control	0.17	0.75	34	45	0.21	0.28	7.4	224
	1300	0.14	0.72	29	42	0.19	0.25	7.2	223
	2600	0.13	0.68	28	38	0.17	0.23	6.7	220
	3900	0.11	0.64	25	36	0.15	0.21	6.3	220
Inoculated	Control	0.23	0.89	40	52	0.30	0.36	9.6	240
	1300	0.21	0.83	35	47	0.27	0.31	9.1	239
	2600	0.19	0.80	32	44	0.24	0.27	7.5	237
	3900	0.17	0.78	31	39	0.23	0.25	7.1	235
LSD		0.008	0.18	1.21	1.03	0.02	0.008	0.03	0.25
F value	Inoculation (df= 1)	712.4*	254*	213.3*	117.4*	7.2*	44.6*	1123*	616.2*
	Insecticide (df=3)	114*	51.6*	18.6*	12.3*	8.4*	3.4	917*	84.3*
	Inoculation × insecticide (df=3)	617.8*	37.6*	11.7*	7.4*	1.8	2.7	87.6*	116.4*

Table 56 Effect of three concentrations of tebuconazole on chlorophyll, leghaemoglobin, N and P content, seed yield and grain protein of pea plants grown in soil inoculated with *Rhizobium* sp. strain MRP1 and without bioinoculant

Treatment	Dose rate (µg/ kg soil)	Leghaemoglobin content [mM (g f.m.)$^{-1}$]	Chlorophyll content (mg/g)	N content (mg/g)		P content (mg/g)		Seed yield (g/plant)	Seed protein (mg/g)
				Root	Shoot	Root	Shoot		
Uninoculated	Control	0.17	0.75	34	45	0.21	0.28	7.4	224
	100	0.12	0.72	30	41	0.18	0.24	6.8	221
	200	0.09	0.69	28	35	0.16	0.22	6.5	220
	300	-	0.65	25	31	0.14	0.19	5.7	219
Inoculated	Control	0.23	0.89	40	52	0.30	0.36	9.6	240
	100	0.18	0.81	36	46	0.25	0.30	8.3	238
	200	0.15	0.79	34	41	0.23	0.28	7.4	236
	300	-	0.76	30	37	0.21	0.25	6.6	235
LSD		0.20	0.13	4.5	4.1	0.02	0.04	3.84	12.6
F value	Inoculation (df= 1)	212.6*	13.4*	100.7*	63.2*	2353.4*	330.4*	862.5*	63.6*
	Fungicides (df=3)	7.0*	0.72	4.5*	6.1*	147.0*	24.7*	28.4*	1.9
	Inoculation × fungicide (df=3)	23.6*	0.38	8.7*	3.7	184.3*	25.3*	76.9*	7.2*

Table 57 Effect of three concentrations of quizalafop-p-ethyl on growth and symbiotic properties of greengram plants grown in soil inoculated with *Bradyrhizobium* sp. (*vigna*) strain MRM6[†] and without bioinoculant

Treatment	Dose rate (µg/ kg soil)	Length/ plant (cm)				Dry biomass (g/ plant)				Nodulation				Total dry biomass (g /plant)	
		Root		Shoot		Root		Shoot		No./ plant		Dry biomass (mg/ plant)			
		50 DAS	80 DAS	50 DAS	80 DAS	50 DAS	80 DAS	50 DAS	80 DAS	50 DAS	80 DAS	50 DAS	80 DAS	50 DAS	80 DAS
Uninoculated	Control	28	30	14	16	0.35	0.47	1.59	2.08	21	17	66	52	2.00	2.60
	40	16	17	7	8	0.14	0.18	0.78	1.19	15	13	41	31	0.96	1.40
	80	13	13	6	7	0.13	0.17	0.66	1.08	13	11	38	25	0.83	1.28
	120	9	10	5	6	0.11	0.16	0.51	0.97	11	10	35	24	0.66	1.15
Inoculated	Control	44	46	29	31	1.14	1.89	4.56	5.73	44	32	151	129	5.85	7.75
	40	23	25	13	14	0.31	0.58	2.44	3.28	19	17	93	82	2.84	3.94
	80	20	22	11	13	0.23	0.48	1.94	2.97	16	15	84	71	2.25	3.52
	120	18	20	10	11	0.17	0.37	1.32	2.77	12	14	78	65	1.57	3.21
LSD		1.7	0.73	0.61	0.71	1.3	1.6	1.3	1.6	2.1	0.76	0.64	1.5	1.4	2.8
F value	Inoculation (df=1)	411*	265*	345*	317*	153*	165*	418*	617*	171*	940*	111*	106*	802*	361*
	Herbicide (df=3)	52.3*	23*	11.5*	16*	43*	37*	74*	112*	63*	112*	36.3*	71.3*	86*	65.3*
	Inoculation × herbicide (df=3)	54.2*	26*	21.4*	13*	21*	12.5*	61*	73*	34*	74.6*	21.3*	19*	61*	54*

[†]Strain MRM6 at 0, 40, 80 and 120 µg L^{-1} of quizalafop-p-ethyl, produced 32, 21, 19 and 12 µg L^{-1} SA; 18, 14, 8 and 5 µg L^{-1} DHBA, 38, 7, 4 and 3 µg ml^{-1} IAA and 21, 22, 24 and 25 µg ml^{-1} EPS, respectively

Table 58 Effect of three concentrations of clodinafop on growth and symbiotic properties of greengram plants grown in soil inoculated with *Bradyrhizobium* sp. (*vigna*) strain MRM6[‡] and without bioinoculant

Treatment	Dose rate (µg/ kg soil)	Length/ plant (cm)				Dry biomass (g/ plant)				Nodulation				Total dry biomass (g /plant)	
		Root		Shoot		Root		Shoot		No./ plant		Dry biomass (mg/ plant)			
		50 DAS	80 DAS	50 DAS	80 DAS	50 DAS	80 DAS	50 DAS	80 DAS	50 DAS	80 DAS	50 DAS	80 DAS	50 DAS	80 DAS
Uninoculated	Control	28	30	14	16	0.35	0.47	1.59	2.08	21	17	66	52	2.00	2.60
	400	14	16	8	11	0.18	0.24	0.84	1.95	19	16	61	47	1.09	2.23
	800	11	12	8	7	0.14	0.20	0.73	1.82	18	14	58	37	0.93	2.06
	1200	10	11	7	6	0.11	0.18	0.63	1.60	16	11	55	32	0.80	1.82
Inoculated	Control	44	46	29	31	1.14	1.89	4.56	5.73	44	32	151	129	5.85	7.75
	400	26	18	16	28	0.52	0.83	2.61	3.61	21	18	116	99	3.25	4.54
	800	23	16	14	24	0.40	0.73	2.09	3.23	18	15	100	87	2.59	4.05
	1200	19	13	11	21	0.29	0.66	1.86	2.93	14	13	89	78	2.24	3.67
LSD		1.5	1.7	1.3	0.9	1.5	1.6	3.1	2.4	1.1	1.4	0.9	1.2	2.3	2.7
F value	Inoculation (df=1)	1019*	319*	819*	388*	114*	53.5*	76.1*	253*	211*	1.6	211*	215*	317.5*	415*
	Herbicide (df=3)	45.3*	27*	44.5*	53.2*	19.5*	44.3*	31.5*	85.3*	44.5*	1.4	65.4*	112.7*	112.2*	119*
	Inoculation × herbicide (df=3)	105.9*	29.5*	109*	35.4*	21.3*	1.5	16.2*	29.1*	5.3	1.3	28.1*	45*	58*	63.5*

[‡]Strain MRM6 at 400, 800 and 1200 µg l^{-1} of clodinafop, produced 21, 19 and 14 µg l^{-1} SA; 15, 11 and 9 µg l^{-1} DHBA; 17, 9 and 7 µg ml^{-1} IAA and 21, 23 and 24 µg ml^{-1} EPS, respectively

Table 59 Effect of three concentrations of fipronil on growth and symbiotic properties of greengram plants grown in soil inoculated with *Bradyrhizobium* sp. (*vigna*) strain MRM6[†] and without bioinoculant

Treatment	Dose rate (µg/ kg soil)	Length/ plant (cm)				Dry biomass (g/ plant)				Nodulation				Total dry biomass (g /plant)	
		Root		Shoot		Root		Shoot		No./ plant		Dry biomass (mg/ plant)			
		50 DAS	80 DAS	50 DAS	80 DAS	50 DAS	80 DAS	50 DAS	80 DAS	50 DAS	80 DAS	50 DAS	80 DAS	50 DAS	80 DAS
Uninoculated	Control	28	30	14	16	0.35	0.47	1.59	2.08	21	17	66	52	2.00	2.60
	200	19	19	13	12	0.17	0.30	1.03	1.34	19	16	58	47	1.26	1.69
	400	14	17	11	11	0.19	0.27	0.92	1.23	17	14	54	41	1.16	1.54
	600	14	15	9	8	0.16	0.23	0.83	1.11	12	10	47	38	1.04	1.38
Inoculated	Control	44	46	29	31	1.14	1.89	4.56	5.73	44	32	151	129	5.85	7.75
	200	32	33	19	21	0.69	0.93	3.70	4.35	28	19	123	97	4.51	5.38
	400	29	30	18	21	0.63	0.81	2.99	4.02	25	16	112	80	3.73	4.91
	600	27	29	16	17	0.59	0.70	2.52	3.67	19	13	103	73	3.21	4.44
LSD		2.1	1.9	1.4	1.7	2.5	2.7	8.6	9.2	0.7	1.2	1.6	1.8	7.4	8.5
F value	Inoculation (df=1)	287*	311*	267*	178*	852*	1603*	971*	1144*	812*	265*	1015*	623*	1131*	1415*
	Insecticide (df=3)	132*	65.2*	73.2*	42.2*	304*	511*	282*	313*	77*	23.4*	117*	63.2*	312*	319*
	Inoculation × insecticide (df=3)	27.3*	23.6*	17.1*	13.3*	228*	344*	321*	221*	63.2*	17.4*	87.2*	36.3*	158.4*	281*

[†]Strain MRM6 at 200, 400 and 600 µg L^{-1} of fipronil, produced 30, 28 and 25 µg L^{-1} SA; 18, 17 and 15 µg L^{-1} DHBA; 27, 15 and 12 µg ml^{-1} IAA and 22, 23 and 26 µg ml^{-1} EPS, respectively

Table 60 Effect of three concentrations of pyriproxyfen on growth and symbiotic properties of greengram plants grown in soil inoculated with *Bradyrhizobium* sp. (*vigna*) strain MRM6[†] and without bioinoculant

Treatment	Dose rate (µg/ kg soil)	Length/ plant (cm)				Dry biomass (g/ plant)				Nodulation				Total dry biomass (g /plant)	
		Root		Shoot		Root		Shoot		No./ plant		Dry biomass (mg/ plant)			
		50 DAS	80 DAS	50 DAS	80 DAS	50 DAS	80 DAS	50 DAS	80 DAS	50 DAS	80 DAS	50 DAS	80 DAS	50 DAS	80 DAS
Uninoculated	Control	28	30	14	16	0.35	0.47	1.59	2.08	21	17	66	52	2.00	2.60
	1300	16	17	10	12	0.10	0.26	0.96	1.33	18	15	54	43	1.12	1.63
	2600	15	16	8	10	0.16	0.22	0.83	1.24	14	12	47	37	1.04	1.49
	3900	11	12	7	9	0.19	0.19	0.76	1.06	12	8	44	32	1.00	1.28
Inoculated	Control	44	46	29	31	1.14	0.19	4.56	5.73	44	32	151	129	5..85	7.75
	1300	30	32	17	19	0.65	0.88	2.92	4.04	22	19	131	111	3.70	5.03
	2600	27	28	15	16	0.50	0.79	1.95	3.63	19	17	127	94	2.58	4.51
	3900	24	26	12	13	0.44	0.70	1.64	3.22	16	13	120	81	2.20	4.00
LSD		1.5	1.3	1.7	2.3	2.8	3.5	4.4	7.4	1.1	1.4	1.3	1.8	7.5	8.7
F value	Inoculation (df=1)	313*	423*	519*	435*	2017*	2728*	577*	5017*	615.4*	328*	719*	854*	2025*	1632*
	Insecticide (df=3)	65.2*	53.2*	78*	73.5*	435*	810*	167*	1213*	93.2*	21.5*	57*	71.4*	418.7*	346.6*
	Inoculation × insecticide (df=3)	13.1*	36.4*	41.4*	31.5*	247.9*	528*	114*	817*	61.2*	13.6*	37.4*	63.2*	238.1*	253.5*

[†]Strain MRM6 at 1300, 2600 and 3900 µg L^{-1} of pyriproxyfen, produced 26, 23 and 21 µg L^{-1} SA; 16, 14 and 12 µg L^{-1} DHBA; 16, 10 and 7 µg ml^{-1} IAA and 22, 25 and 26 µg ml^{-1} EPS, respectively

Table 61 Effect of three concentrations of tebuconazole on growth and symbiotic properties of greengram plants grown in soil inoculated with *Bradyrhizobium* sp. (*vigna*) strain MRM6[†] and without bioinoculant

Treatment	Dose rate (µg/ kg soil)	Length/ plant (cm)				Dry biomass (g/ plant)				Nodulation				Total dry biomass (g /plant)	
		Root		Shoot		Root		Shoot		No./ plant		Dry biomass (mg/ plant)			
		50 DAS	80 DAS	50 DAS	80 DAS	50 DAS	80 DAS	50 DAS	80 DAS	50 DAS	80 DAS	50 DAS	80 DAS	50 DAS	80 DAS
Uninoculated	Control	28	30	14	16	0.35	0.47	1.59	2.08	21	17	66	52	2.00	2.60
	100	22	24	13	14	0.29	0.37	1.18	1.34	16	13	50	44	1.52	1.76
	200	18	20	11	12	0.24	0.32	1.06	1.20	12	9	44	38	1.35	1.56
	300	18	19	9	10	0.23	0.27	0.95	1.08	10	8	40	32	1.22	1.38
Inoculated	Control	44	46	29	31	1.14	1.89	4.56	5.73	44	32	151	129	5.85	7.75
	100	34	36	23	25	0.81	0.96	3.98	4.96	34	27	139	114	4.93	6.03
	200	31	33	18	21	0.78	0.89	3.56	4.63	29	21	129	95	4.47	5.62
	300	28	29	17	19	0.72	0.82	2.86	4.29	24	15	120	82	3.70	5.19
LSD		2.3	1.4	1.4	1.2	2.4	2.2	3.6	2.7	1.2	0.41	0.63	0.91	1.9	2.8
F value	Inoculation (df=1)	648*	361*	847*	615*	119.2*	65.3*	83.4*	217*	207*	2.4	211*	324*	408*	318*
	Fungicides (df=3)	53.3*	24.2*	65.4*	'37*	25.6*	36.5*	52.3*	83.4*	47.4*	1.7	61.3*	123.4*	121.2*	134.4*
	Inoculation × fungicide (df=3)	103*	29.3*	73.3*	29*	3.3	1.8	21*	31.6*	5.2	1.8	27.2*	45*	64.4*	76.2*

[†]Strain MRM6 at 100, 200 and 300 µg L^{-1} of tebuconazole, produced 24, 21 and 18 µg L^{-1} SA; 15, 13 and 10 µg L^{-1} DHBA; 7, 6 and 4 µg ml^{-1} IAA and 23, 25 and 27 µg ml^{-1} EPS, respectively

Table 62 Effect of three concentrations of quizalafop-p-ethyl on biological and chemical properties of greengram plants grown in soil inoculated with *Bradyrhizobium* sp. (*vigna*) strain MRM6 and without bioinoculant

Treatment	Dose rate (µg/ kg soil)	Leghaemoglobin content [mM (g f.m.)$^{-1}$]	Chlorophyll content (mg/g)	N content (mg/g)		P content (mg/g)		Seed yield (g/plant)	Seed protein (mg/g)
				Root	Shoot	Root	Shoot		
Uninoculated	Control	0.08	0.82	36	50	0.27	0.36	7.4	261
	40	0.06	0.74	27	38	0.18	0.28	3.6	235
	80	0.04	0.72	25	32	0.16	0.25	3.2	233
	120	0.03	0.68	20	28	0.13	0.23	2.8	230
Inoculated	Control	0.11	0.94	45	61	0.32	0.41	10.9	269
	40	0.08	0.79	29	46	0.24	0.30	7.4	249
	80	0.06	0.77	27	42	0.22	0.27	6.1	245
	120	0.04	0.74	24	38	0.19	0.24	5.5	241
LSD		0.005	0.04	1.1	1.5	0.04	0.03	0.16	1.7
F value	Inoculation (df= 1)	453.6*	31.4*	157.4*	217.3*	417*	379*	188.3*	219.3*
	Herbicide (df=3)	2.4	1.3	103.2*	19.2*	137.5*	72.3*	117.4*	96.2*
	Inoculation × herbicide (df=3)	54.3*	1.1	19.2*	21.3*	65.1*	42.4*	43.5*	34.4*

Table 63 Effect of three concentrations of clodinafop on biological and chemical properties of greengram plants grown in soil inoculated with *Bradyrhizobium* sp. (*vigna*) strain MRM6 and without bioinoculant

Treatment	Dose rate (µg/ kg soil)	Leghaemoglobin content [mM (g f.m.)$^{-1}$]	Chlorophyll content (mg/g)	N content (mg/g)		P content (mg/g)		Seed yield (g/plant)	Seed protein (mg/g)
				Root	Shoot	Root	Shoot		
Uninoculated	Control	0.08	0.82	36	50	0.27	0.36	7.4	261
	400	0.07	0.80	35	48	0.26	0.34	6.4	258
	800	0.06	0.78	32	45	0.25	0.31	6.1	255
	1200	0.05	0.75	30	42	0.23	0.29	5.3	251
Inoculated	Control	0.11	0.94	45	61	0.32	0.41	10.9	269
	400	0.10	0.92	42	58	0.29	0.38	10.2	267
	800	0.09	0.89	39	55	0.27	0.35	9.5	263
	1200	0.08	0.87	37	55	0.25	0.32	9.1	260
LSD		0.007	0.03	1.4	1.8	0.03	0.04	0.09	1.2
F value	Inoculation (df=1)	443.6*	20.2*	145.6*	168.7*	344.2*	378*	115.8*	194.1*
	Herbicide (df=3)	1.9	1.2	76.6*	16.2*	127.7*	45.7*	91.4*	112.3*
	Inoculation × herbicide (df=3)	39.2*	1.5	15.2*	15.2*	41.3*	35.4*	27.3*	21.6*

Table 64 Effect of three concentrations of fipronil on biological and chemical properties of greengram plants grown in soil inoculated with *Bradyrhizobium* sp. (*vigna*) strain MRM6 and without bioinoculant

Treatment	Dose rate (µg/ kg soil)	Leghaemoglobin content [mM (g f.m.)$^{-1}$]	Chlorophyll content (mg/g)	N content (mg/g)		P content (mg/g)		Seed yield (g/plant)	Seed protein (mg/g)
				Root	Shoot	Root	Shoot		
Uninoculated	Control	0.08	0.82	36	50	0.27	0.36	7.4	261
	200	0.07	0.77	33	44	0.25	0.35	6.2	256
	400	0.06	0.74	29	42	0.23	0.33	5.7	253
	600	0.05	0.72	24	39	0.21	0.31	4.6	248
Inoculated	Control	0.11	0.94	45	61	0.32	0.41	10.9	269
	200	0.09	0.91	38	57	0.28	0.36	9.2	265
	400	0.08	0.85	36	54	0.25	0.34	8.7	263
	600	0.07	0.82	31	51	0.23	0.31	8.2	261
LSD		0.006	0.04	1.3	2.2	0.07	0.05	0.03	1.8
F value	Inoculation (df= 1)	345*	4.4*	513*	49.2*	847.5*	942*	291.5*	27*
	Insecticide (df=3)	9.1*	0.7	317.4*	33.3*	412*	79.5*	53.5*	14*
	Inoculation × insecticide (df=3)	43.6*	0.6	64.3*	12.5*	85.9*	129.4*	26.7*	4.3*

Table 65 Effect of three concentrations of pyriproxyfen on biological and chemical properties of greengram plants grown in soil inoculated with *Bradyrhizobium* sp. (*vigna*) strain MRM6 and without bioinoculant

Treatment	Dose rate (µg/ kg soil)	Leghaemoglobin content [mM (g f.m.)$^{-1}$]	Chlorophyll content (mg/g)	N content (mg/g)		P content (mg/g)		Seed yield (g/plant)	Seed protein (mg/g)
				Root	Shoot	Root	Shoot		
Uninoculated	Control	0.08	0.82	36	50	0.27	0.36	7.4	261
	1300	0.07	0.76	31	43	0.22	0.31	5.5	251
	2600	0.06	0.74	27	37	0.19	0.29	5.1	247
	3900	0.04	0.71	23	34	0.17	0.27	4.5	244
Inoculated	Control	0.11	0.94	45	61	0.32	0.41	10.9	269
	1300	0.09	0.87	35	54	0.27	0.34	8.5	257
	2600	0.08	0.84	32	49	0.24	0.32	8.2	254
	3900	0.07	0.82	29	45	0.21	0.29	7.8	252
LSD		0.008	0.17	1.4	1.5	0.005	0.007	0.03	1.2
F value	Inoculation (df= 1)	425.2*	1578.8*	26.7*	155.3*	172.5*	33.3*	999.6*	80.4*
	Insecticide (df=3)	38.5*	126.7*	3.0	10.2*	64.2*	2.6	66.3*	12.6*
	Inoculation × insecticide (df=3)	43.7*	181.1*	1.3	1.3	16.1*	2.7	155.8*	9.9*

Table 66 Effect of three concentrations of tebuconazole on biological and chemical properties of greengram plants grown in soil inoculated with *Bradyrhizobium* sp. (*vigna*) strain MRM6 and without bioinoculant

Treatment	Dose rate (µg/ kg soil)	Leghaemoglobin content [mM (g f.m.)$^{-1}$]	Chlorophyll content (mg/g)	N content (mg/g)		P content (mg/g)		Seed yield (g/plant)	Seed protein (mg/g)
				Root	Shoot	Root	Shoot		
Uninoculated	Control	0.08	0.82	36	50	0.27	0.36	7.4	261
	100	0.07	0.75	29	41	0.21	0.29	4.8	246
	200	0.05	0.72	25	37	0.18	0.26	4.3	244
	300	0.04	0.70	27	35	0.17	0.24	3.8	241
Inoculated	Control	0.11	0.94	45	61	0.32	0.41	10.9	269
	100	0.08	0.82	34	53	0.25	0.33	8.5	255
	200	0.07	0.78	30	45	0.23	0.31	7.1	253
	300	0.05	0.76	27	41	0.19	0.28	6.8	248
LSD		0.006	0.05	1.3	2.2	0.06	0.04	0.02	2.3
F value	Inoculation (df= 1)	417*	7.5*	519.5*	62.4*	818*	1228*	307.5*	107*
	Fungicides (df=3)	15.9*	0.8	287.4*	42.3*	412.8*	86.3*	65.3*	15.3*
	Inoculation × fungicide (df=3)	24.6*	0.6	67.7*	12.2*	106.2*	22.8*	21.3*	4.4*

Table 67 Effect of three concentrations of quizalafop-p-ethyl on growth and nodulation of lentil plants grown in soil inoculated with *Rhizobium* sp. strain MRL3[†] and without bioinoculant

Treatment	Dose rate (µg/ kg soil)	Length/ plant (cm)				Dry biomass (g/ plant)				Nodulation				Total dry biomass (g /plant)	
		Root		Shoot		Root		Shoot		No./ plant		Dry biomass (mg/ plant)			
		90 DAS	120 DAS	90 DAS	120 DAS	90 DAS	120 DAS	90 DAS	120 DAS	90 DAS	120 DAS	90 DAS	120 DAS	90 DAS	120 DAS
Uninoculated	Control	17	21	20	31	366	536	1076	1966	19	38	30	74	1.47	2.57
	40	8	16	13	18	220	260	600	1166	-	26	-	36	0.82	1.46
	80	6	13	9	12	173	220	486	833	-	21	-	31	0.66	1.08
	120	3	11	4	10	120	160	380	730	-	14	-	27	0.50	0.92
Inoculated	Control	20	24	28	34	570	706	2130	3433	28	42	72	84	2.77	4.23
	40	12	19	16	19	213	477	1130	1800	27	33	25	48	1.37	2.33
	80	9	17	12	14	173	445	730	1533	20	31	18	39	0.92	2.02
	120	5	13	5	11	126	323	500	1233	18	16	12	30	0.64	1.59
LSD		1.8	1.2	1.4	2.2	3.2	3.0	7.5	9.2	1.3	1.9	0.8	1.3	7.4	10.5
F value	Inoculation (df=1)	639*	346*	428*	517*	3487*	2182*	518*	1649*	613*	872.4*	712*	544*	6912*	2019*
	Herbicide (df=3)	171.4*	35.3*	125*	64.8*	829*	239*	105*	412*	85.6*	65.4*	105.3*	104.1*	1306*	276*
	Inoculation × herbicide (df=3)	25.4*	27.6*	51.4*	21.2*	539*	472*	214*	127*	27.4*	32.2*	91.5*	34.6*	1203*	519.2*

[†]Strain MRL3 at 0, 40, 80 and 120 µg L^{-1} of quizalafop-p-ethyl, produced 29, 21, 17 and 15 µg L^{-1} SA; 21, 16, 10 and 9 µg L^{-1} DHBA, 37, 27, 23 and 20 µg ml^{-1} IAA and 18, 20, 21 and 24 µg ml^{-1} EPS, respectively

Table 68 Effect of three concentrations of clodinafop on growth and nodulation of lentil plants grown in soil inoculated with *Rhizobium* sp. strain MRL3[†] and without bioinoculant

Treatment	Dose rate (µg/ kg soil)	Length/ plant (cm)				Dry biomass (g/ plant)				Nodulation				Total dry biomass (g /plant)	
		Root		Shoot		Root		Shoot		No./ plant		Dry biomass (mg/ plant)			
		90 DAS	120 DAS	90 DAS	120 DAS	90 DAS	120 DAS	90 DAS	120 DAS	90 DAS	120 DAS	90 DAS	120 DAS	90 DAS	120 DAS
Uninoculated	Control	17	21	20	31	0.37	0.54	1.08	197	19	38	30	74	1.47	2.57
	400	16	21	19	30	0.33	0.50	0.99	1.87	18	37	28	69	1.35	2.44
	800	13	18	17	27	0.27	0.39	0.89	1.67	17	31	24	62	1.18	2.12
	1200	9	16	15	22	0.22	0.36	0.78	1.53	12	28	21	51	1.02	1.94
Inoculated	Control	20	24	28	34	0.57	0.71	2.13	3.43	28	42	72	84	2.77	4.23
	400	19	23	24	32	0.50	0.68	1.66	3.30	27	40	63	80	2.22	4.06
	800	17	21	22	28	0.38	0.65	1.60	2.93	24	36	54	74	2.04	3.66
	1200	14	19	19	24	0.28	0.59	1.30	2.77	19	24	43	69	62	3.43
LSD		1.7	1.5	1.8	2.2	2.7	2.4	7.1	8.3	0.5	0.8	1.2	0.9	6.5	6.9
F value	Inoculation (df=1)	609*	667*	242*	714.6*	4717*	549*	637*	190.2*	2680*	455*	1145*	1616*	1456*	1584*
	Herbicide (df=3)	43.3*	131.4*	28.4*	117.5*	968*	53.6*	85*	87.4*	235*	209*	475*	353*	295.3*	568*
	Inoculation × herbicide (df=3)	21.2*	65.5*	17*	27.7*	487*	31.3*	118.6*	14.2*	69*	29.5*	205*	104.7*	804*	102*

[†]Strain MRL3 at 400, 800 and 1200 µg L^{-1} of clodinafop, produced 25, 22 and 17 µg L^{-1} SA; 17, 14 and 10 µg L^{-1} DHBA; 33, 27 and 22 µg ml^{-1} IAA and 19, 20 and 22 µg ml^{-1} EPS, respectively

Table 69 Effect of three concentrations of fipronil on growth and nodulation of lentil plants grown in soil inoculated with *Rhizobium* sp. strain MRL3[+] and without bioinoculant

Treatment	Dose rate (µg/ kg soil)	Length/ plant (cm)				Dry biomass (g/ plant)				Nodulation				Total dry biomass (g /plant)	
		Root		Shoot		Root		Shoot		No./ plant		Dry biomass (mg/ plant)			
		90 DAS	120 DAS	90 DAS	120 DAS	90 DAS	120 DAS	90 DAS	120 DAS	90 DAS	120 DAS	90 DAS	120 DAS	90 DAS	120 DAS
Uninoculated	Control	17	21	20	31	0.37	0.54	1.08	1.97	19	38	30	74	1.47	2.57
	200	17	17	17	25	0.30	0.42	0.88	1.77	18	34	25	60	1.20	2.25
	400	12	15	14	22	0.23	0.33	0.75	1.66	13	28	22	49	1.01	2.04
	600	9	12	11	17	0.19	0.30	0.67	1.50	12	17	19	38	0.88	1.84
Inoculated	Control	18	24	28	34	0.57	0.71	2.13	3.43	28	42	72	84	2.77	4.23
	200	16	21	20	28	0.40	0.66	1.67	3.20	27	33	52	74	2.12	3.86
	400	14	17	18	22	0.35	0.63	1.50	2.77	18	30	41	67	1.89	3.46
	600	10	15	16	19	0.29	0.55	1.33	2.37	18	19	35	61	1.66	2.98
LSD		2.2	2.5	2.1	2.7	0.43	0.28	0.24	0.57	3.2	4.1	0.08	0.27	0.44	0.83
F value	Inoculation (df=1)	1206*	768*	18.2*	501.6*	687*	162*	19.4*	240*	204*	447*	18.6*	302.2*	114.9*	365*
	Insecticide (df=3)	309.4*	28.3*	1.4	48.2*	57.7*	40*	18.7*	82.4*	7.8*	31.6*	3.8*	30.5*	35.4*	24.2*
	Inoculation × insecticide (df=3)	94*	52.4*	0.7	38.5*	21.2*	8.5*	1.4	31.5*	10.1*	423*	1.2	8.2*	12.5*	26.2*

[+]Strain MRL3 at 200, 400 and 600 µg L^{-1} of fipronil, produced 27, 21 and 18 µg L^{-1} SA; 18, 16 and 13 µg L^{-1} DHBA; 31, 28 and 24 µg ml^{-1} IAA and 19, 22 and 23 µg ml^{-1} EPS respectively

Table 70 Effect of three concentrations of pyriproxyfen on growth and nodulation of lentil plants grown in soil inoculated with *Rhizobium* sp. strain MRL3[+] and without bioinoculant

Treatment	Dose rate (µg/ kg soil)	Length/ plant (cm)				Dry biomass (g/ plant)				Nodulation				Total dry biomass (g /plant)	
		Root		Shoot		Root		Shoot		No./ plant		Dry biomass (mg/ plant)			
		90 DAS	120 DAS	90 DAS	120 DAS	90 DAS	120 DAS	90 DAS	120 DAS	90 DAS	120 DAS	90 DAS	120 DAS	90 DAS	120 DAS
Uninoculated	Control	17	31	20	21	0.37	0.54	1.08	1.97	19	38	30	74	1.47	2.57
	1300	13	24	19	20	0.25	0.40	0.80	1.66	18	32	20	51	1.07	2.16
	2600	11	20	15	13	0.21	0.31	0.71	1.43	13	29	15	45	0.93	1.78
	3900	8	16	11	12	0.17	0.26	0.61	1.30	11	23	12	34	0.78	1.59
Inoculated	Control	20	24	28	34	0.57	0.71	2.13	3.43	28	42	72	84	2.77	4.23
	1300	17	20	23	27	0.34	0.66	1.53	2.73	27	30	67	63	1.93	4.12
	2600	14	16	18	22	0.29	0.57	1.23	2.27	21	25	35	55	1.56	2.90
	3900	12	14	16	18	0.27	0.46	1.03	1.93	19	20	27	50	1.32	2.44
LSD		2.3	1.4	1.7	2.4	0.18	0.25	0.67	0.85	3.6	4.3	1.5	0.9	2.8	4.6
F value	Inoculation (df=1)	649.5*	362.4*	713.7*	714.6*	567.4*	379.7*	127*	1122*	316*	217.6*	982*	602*	167*	153.6*
	Insecticide (df=3)	127.4*	45.3*	325*	117.4*	134.5*	29*	52.1*	423.2*	23.4*	103.7*	209*	240.3*	63.4*	108.6*
	Inoculation × insecticide (df=3)	42.2*	18.6*	74.5*	63.2*	428.3*	21.6*	19.2*	168.4*	15.5*	17.3	71.4*	55.8*	25.3*	76.4

[+]Strain MRL3 at 1300, 2600 and 3900 µg L^{-1} of pyriproxyfen, produced 25, 24 and 21 µg L^{-1} SA; 14, 12 and 9 µg L^{-1} DHBA; 28, 23 and 20 µg ml^{-1} IAA and 19, 21 and 24 µg ml^{-1} EPS, respectively

Table 71 Effect of three concentrations of tebuconazole on growth and nodulation of lentil plants grown in soil inoculated with *Rhizobium* sp. strain MRL3[*] and without bioinoculant

Treatment	Dose rate (µg/ kg soil)	Length/ plant (cm)				Dry biomass (g/ plant)				Nodulation				Total dry biomass (g /plant)	
		Root		Shoot		Root		Shoot		No./ plant		Dry biomass (mg/ plant)			
		90 DAS	120 DAS	90 DAS	120 DAS	90 DAS	120 DAS	90 DAS	120 DAS	90 DAS	120 DAS	90 DAS	120 DAS	90 DAS	120 DAS
Uninoculated	Control	17	21	20	31	0.37	0.54	1.08	1.97	19	38	30	74	1.47	2.57
	100	13	17	19	20	0.23	0.37	0.69	1.50	15	27	14	43	0.97	1.91
	200	10	15	14	15	0.16	0.25	0.60	1.27	-	25	-	37	0.76	1.55
	300	7	13	11	12	0.13	0.18	0.52	0.97	-	13	-	29	0.65	1.18
Inoculated	Control	20	24	28	34	0.57	0.71	2.13	3.43	28	42	72	84	2.77	4.23
	100	16	20	21	22	0.30	0.53	1.40	2.70	24	40	33	56	1.74	3.29
	200	12	17	18	17	0.24	0.50	1.10	2.30	22	35	30	49	1.37	2.85
	300	10	14	14	14	0.19	0.39	0.90	1.97	19	22	24	45	1.12	2.41
LSD		1.5	1.1	1.3	1.5	1.8	2.1	1.2	1.5	1.6	1.4	0.95	0.78	1.3	1.7
F value	Inoculation (df=1)	520*	311*	402*	419*	210*	215*	303.5*	710*	234*	1015*	163*	106*	1217*	423*
	Fungicides (df=3)	86*	37*	74*	21.6*	38.9*	43.5*	51.4*	241.3*	45.4*	134*	41.4*	65.4*	231.6*	14.5*
	Inoculation × fungicide (df=3)	43*	29*	61.3*	16.3*	44.6*	17.3*	31*	45.2*	73*	33.2*	19.2*	18.5*	112.4*	27.9*

[*]Strain MRL3 at 100, 200 and 300 µg L^{-1} of tebuconazole, produced 20, 16 and 14 µg L^{-1} SA; 14, 12 and 10 µg L^{-1} DHBA; 21, 17 and 15 µg ml^{-1} IAA and 20, 20 and 22 µg ml^{-1} EPS, respectively

Table 72 Effect of three concentrations of quizalafop-p-ethyl on chlorophyll, leghaemoglobin, N and P content, seed yield and grain protein of lentil plants grown in soil inoculated with *Rhizobium* sp. strain MRL3 and without bioinoculant

Treatment	Dose rate (µg/ kg soil)	Leghaemoglobin content [mM (g f.m.)$^{-1}$]	Chlorophyll content (mg/g)	N content (mg/g)		P content (mg/g)		Seed yield (g/plant)	Seed protein (mg/g)
				Root	Shoot	Root	Shoot		
Uninoculated	Control	0.12	0.32	17	45	0.21	0.28	3.0	232
	40	-	0.25	12	38	0.16	0.21	1.3	221
	80	-	0.21	10	36	0.14	0.19	0.8	216
	120	-	0.19	9	34	0.12	0.17	0.6	212
Inoculated	Control	0.15	0.38	21	49	0.29	0.34	4.1	245
	40	0.09	0.27	17	42	0.23	0.28	2.2	229
	80	0.08	0.24	14	38	0.21	0.26	1.9	225
	120	0.06	0.20	11	36	0.19	0.23	1.6	221
LSD		0.004	0.12	1.4	1.8	0.004	0.007	0.06	3.6
F value	Inoculation (df= 1)	416.2*	256.1*	164.2*	1217.2*	287.9*	1141*	170.1*	510.2*
	Herbicide (df=3)	74.5*	108.4*	43.2*	512.3*	67.2*	217*	28.1*	127.5*
	Inoculation × herbicide (df=3)	152.1*	11.4*	12.5*	117.4*	38.1*	67.5*	11.8*	417.4*

Table 73 Effect of three concentrations of clodinafop on chlorophyll, leghaemoglobin, N and P content, seed yield and grain protein of lentil plants grown in soil inoculated with *Rhizobium* sp. strain MRL3 and without bioinoculant

Treatment	Dose rate (µg/ kg soil)	Leghaemoglobin content [mM (g f.m.)$^{-1}$]	Chlorophyll content (mg/g)	N content (mg/g)		P content (mg/g)		Seed yield (g/plant)	Seed protein (mg/g)
				Root	Shoot	Root	Shoot		
Uninoculated	Control	0.12	0.32	17	45	0.21	0.28	3.0	232
	400	0.11	0.31	16	43	0.20	0.26	2.6	229
	800	0.09	0.29	15	41	0.19	0.25	2.2	226
	1200	0.08	0.27	13	40	0.17	0.23	2.0	222
Inoculated	Control	0.15	0.38	21	49	0.29	0.34	4.1	245
	400	0.13	0.36	20	47	0.27	0.33	3.9	242
	800	0.12	0.33	19	45	0.26	0.31	3.4	238
	1200	0.10	0.31	17	43	0.24	0.28	3.1	236
LSD		0.003	0.05	1.3	2.2	0.004	0.005	0.06	2.5
F value	Inoculation (df=1)	144.2*	652*	1029*	984.2*	407.3*	225.5*	2550*	456.8*
	Herbicide (df=3)	16*	101*	186*	127.4*	87.2*	45.2*	317.5*	108.2*
	Inoculation × herbicide (df=3)	5.1*	36.1*	42.3*	26.5*	18.4*	12.5*	78.1*	51.4*

Table 74 Effect of three concentrations of fipronil on chlorophyll, leghaemoglobin, N and P content, seed yield and grain protein of soil inoculated with *Rhizobium* sp. strain MRL3 and without bioinoculant

Treatment	Dose rate (µg/ kg soil)	Leghaemoglobin content [mM (g f.m.)$^{-1}$]	Chlorophyll content (mg/g)	N content (mg/g)		P content (mg/g)		Seed yield (g/plant)	Seed protein (mg/g)
				Root	Shoot	Root	Shoot		
Uninoculated	Control	0.12	0.32	17	45	0.21	0.28	3.0	232
	200	0.10	0.30	15	42	0.18	0.24	2.2	227
	400	0.08	0.28	13	39	0.17	0.23	1.8	223
	600	0.07	0.25	11	37	0.15	0.21	1.6	219
Inoculated	Control	0.15	0.38	21	49	0.29	0.34	4.1	245
	200	0.12	0.35	19	46	0.26	0.32	3.5	239
	400	0.11	0.32	18	44	0.24	0.29	3.1	234
	600	0.09	0.30	16	41	0.22	0.27	2.9	231
LSD		0.018	0.06	1.06	1.2	0.006	0.007	0.05	7.3
F value	Inoculation (df=1)	553*	302.5*	342*	164.6*	62.6*	737.4*	108.9*	1265*
	Insecticide (df=3)	69*	72.2*	24.7*	18.5*	16.4*	145*	17.5*	927.1*
	Inoculation × insecticide (df=3)	42.5*	33.1*	3.2	7.8*	1.2	63.5*	9.0*	105*

Table 75 Effect of three concentrations of pyriproxyfen on chlorophyll, leghaemoglobin, N and P content, seed yield and grain protein of lentil plants grown in soil inoculated with *Rhizobium* sp. strain MRL3 and without bioinoculant

Treatment	Dose rate (µg/ kg soil)	Leghaemoglobin content [mM (g f.m.)$^{-1}$]	Chlorophyll content (mg/g)	N content (mg/g)		P content (mg/g)		Seed yield (g/plant)	Seed protein (mg/g)
				Root	Shoot	Root	Shoot		
Uninoculated	Control	0.12	0.32	17	45	0.21	0.28	3.0	232
	1300	0.09	0.29	14	41	0.17	0.22	1.8	225
	2600	0.08	0.26	12	38	0.16	0.19	1.5	222
	3900	0.06	0.25	10	36	0.14	0.17	1.3	217
Inoculated	Control	0.15	0.38	21	49	0.29	0.34	4.1	245
	1300	0.11	0.31	18	45	0.25	0.30	2.9	237
	2600	0.09	0.29	16	42	0.23	0.28	2.5	235
	3900	0.08	0.27	14	39	0.21	0.25	2.2	230
LSD		0.007	0.03	0.34	0.27	0.05	0.02	0.21	7.6
F value	Inoculation (df=1)	945.3*	512.3*	265.3*	578.5*	914*	1142*	128.6*	148.7*
	Insecticide (df=3)	236.2*	407.4*	48.3*	104.2*	25.7*	205.1*	37*	67.4*
	Inoculation × insecticide (df=3)	82.5*	61.7*	112*	625.4*	144*	87.2*	13.4*	19.6*

Table 76 Effect of three concentrations of tebuconazole on chlorophyll, leghaemoglobin, N and P content, seed yield and grain protein of lentil plants grown in soil inoculated with *Rhizobium* sp. strain MRL3 and without bioinoculant

Treatment	Dose rate (µg/ kg soil)	Leghaemoglobin content [mM (g f.m.)$^{-1}$]	Chlorophyll content (mg/g)	N content (mg/g)		P content (mg/g)		Seed yield (g/plant)	Seed protein (mg/g)
				Root	Shoot	Root	Shoot		
Uninoculated	Control	0.12	0.32	17	45	0.21	0.28	3.0	232
	100	0.08	0.28	13	39	0.17	0.24	1.2	223
	200	-	0.25	11	37	0.15	0.22	1.0	219
	300	-	0.22	9	35	0.13	0.19	0.9	216
Inoculated	Control	0.15	0.38	21	49	0.29	0.34	4.1	245
	100	0.11	0.30	17	43	0.24	0.29	2.6	236
	200	0.08	0.28	15	41	0.22	0.27	2.2	231
	300	0.07	0.25	12	38	0.21	0.24	1.9	228
LSD		0.006	0.009	1.3	1.4	0.06	0.008	0.07	1.4
F value	Inoculation (df= 1)	43.6*	617.5*	416.3*	620*	318.7*	217.4*	327.4*	219.7*
	Fungicides (df=3)	1.5	3.8	87.4*	163*	33.7*	127.2*	112.5*	144.2*
	Inoculation × fungicide (df=3)	1.2	71.4*	37.5*	82*	79.5*	32.4*	38.6*	63.5*

Table 77 Effect of three concentrations of quizalafop-p-ethyl on growth and symbiotic properties of greengram plants grown in soil inoculated with *Pseudomonas aeruginosa* strain PS1[†] and without bioinoculant

Treatment	Dose rate (µg/ kg soil)	Length/ plant (cm)				Dry biomass (g/ plant)				Nodulation				Total dry biomass (g /plant)	
		Root		Shoot		Root		Shoot		No./ plant		Dry biomass (mg/ plant)			
		50 DAS	80 DAS	50 DAS	80 DAS	50 DAS	80 DAS	50 DAS	80 DAS	50 DAS	80 DAS	50 DAS	80 DAS	50 DAS	80 DAS
Uninoculated	Control	28	30	14	16	0.35	0.47	1.59	2.08	21	17	66	52	2.00	2.60
	40	16	17	7	8	0.14	0.18	0.78	1.19	15	13	41	31	0.96	1.40
	80	13	13	6	7	0.13	0.17	0.66	1.08	13	11	38	25	0.83	1.28
	120	9	10	5	6	0.11	0.16	0.51	0.97	11	10	35	24	0.66	1.15
Inoculated	Control	33	36	30	32	1.76	2.56	8.70	11.66	41	36	399	316	10.86	14.54
	40	28	30	25	28	1.20	1.66	3.93	5.56	30	25	111	95	5.24	7.32
	80	24	24	23	24	0.86	1.40	3.26	4.93	28	23	95	77	4.22	6.41
	120	19	21	17	19	0.80	1.13	2.50	4.40	23	18	77	59	3.38	5.59
LSD		1.21	1.60	1.12	0.94	1.6	2.3	1.7	2.8	1.7	1.2	1.6	2.5	2.5	3.6
F value	Inoculation (df=1)	541.5*	630.3*	3623.8*	1633*	243*	1108*	547*	1115*	388*	987*	122*	147*	912*	1113*
	Herbicide (df=3)	292.5*	352.3*	307.7*	429.0*	125*	136*	187*	307*	83.2*	69*	65.3*	74*	127*	235.7*
	Inoculation × herbicide (df=3)	14.5*	13.38*	25.3*	37.5*	24*	19.8*	63*	106*	42*	53*	37*	35*	64.4*	84*

[†]Strain PS1 at 0, 40, 80 and 120 µg L^{-1} of quizalafop-p-ethyl, produced 41, 38, 32 and 27 µg L^{-1} SA; 21, 19, 15 and 11 µg L^{-1} DHBA, 39, 9, 5 and 4 µg ml^{-1} IAA and 18, 21, 23 and 25 µg ml^{-1} EPS, respectively

Table 78 Effect of three concentrations of clodinafop on growth and symbiotic properties of greengram plants grown in soil inoculated with *Pseudomonas aeruginosa* strain PS1[†] and without bioinoculant

Treatment	Dose rate (µg/ kg soil)	Length/ plant (cm)				Dry biomass (mg/ plant)				Nodulation				Total dry biomass (g /plant)	
		Root		Shoot		Root		Shoot		No./ plant		Dry biomass (mg/ plant)			
		50 DAS	80 DAS	50 DAS	80 DAS	50 DAS	80 DAS	50 DAS	80 DAS	50 DAS	80 DAS	50 DAS	80 DAS	50 DAS	80 DAS
Uninoculated	Control	28	30	14	16	0.35	0.47	1.59	2.08	21	17	66	52	2.00	2.60
	400	14	16	8	11	0.18	0.24	0.84	1.95	19	16	61	47	1.09	2.23
	800	11	12	8	7	0.14	0.20	0.73	1.82	18	14	58	37	0.93	2.06
	1200	10	11	7	8	0.11	0.18	0.63	1.60	16	11	55	32	0.80	1.82
Inoculated	Control	33	36	30	32	1.76	2.56	8.70	11.66	41	36	399	316	10.86	14.54
	400	30	31	27	29	1.56	2.03	6.10	8.10	48	34	266	205	7.93	10.36
	800	23	25	26	27	1.43	1.73	5.16	7.23	46	27	206	168	6.80	9.13
	1200	22	27	26	26	1.16	1.46	3.86	6.30	41	18	153	126	5.17	7.89
LSD		1.7	1.4	1.6	1.3	1.6	2.3	1.5	2.7	1.6	1.8	1.6	2.1	3.5	4.1
F value	Inoculation (df=1)	836.3*	511*	947*	712*	206*	212*	29.7*	619*	294.3*	719*	184*	122*	244*	183*
	Herbicide (df=3)	342.2*	120*	203.4*	244.5*	54*	64*	23*	103*	37*	36.5*	83*	30.2*	112.5*	37.5*
	Inoculation × herbicide (df=3)	89*	29.6*	64.5*	75*	19*	1.4	42.4*	70.5*	31*	17.4*	1.4	7.9*	54.6*	25.3*

[†]Strain PS1 at 400, 800 and 1200 µg L^{-1} of clodinafop, produced 39, 34 and 29 µg L^{-1} SA; 10, 8 and 6 µg L^{-1} DHBA; 25, 13 and 9 µg ml^{-1} IAA and 22, 24 and 25 µg ml^{-1} EPS, respectively

Table 79 Effect of three concentrations of fipronil on growth and symbiotic properties of greengram plants grown in soil inoculated with *Pseudomonas aeruginosa* strain PS1[†] and without bioinoculant

Treatment	Dose rate (µg/ kg soil)	Length/ plant (cm)				Dry biomass (g/ plant)				Nodulation				Total dry biomass (g /plant)	
		Root		Shoot		Root		Shoot		No./ plant		Dry biomass (mg/ plant)			
		50 DAS	80 DAS	50 DAS	80 DAS	50 DAS	80 DAS	50 DAS	80 DAS	50 DAS	80 DAS	50 DAS	80 DAS	50 DAS	80 DAS
Uninoculated	Control	28	30	14	16	0.35	0.47	1.59	2.08	21	17	66	52	2.00	2.60
	200	19	19	17	12	0.19	0.30	1.03	1.34	19	16	58	47	1.26	1.69
	400	14	17	16	11	0.17	0.27	0.92	1.23	17	14	54	41	1.16	1.54
	600	14	15	13	8	0.16	0.23	0.83	1.11	12	10	47	38	1.04	1.38
Inoculated	Control	33	36	30	32	1.76	2.56	8.70	11.66	41	36	399	316	10.86	14.54
	200	32	34	29	29	1.26	1.93	6.20	7.90	28	23	214	161	7.68	9.99
	400	32	33	27	29	1.20	1.63	5.33	6.93	31	19	184	143	6.71	8.70
	600	28	27	29	28	0.96	1.33	4.50	5.50	25	17	132	122	5.59	6.95
LSD		1.6	2.1	2.3	2.5	3.3	2.8	5.7	6.2	0.8	1.5	0.6	1.2	7.5	7.8
F value	Inoculation (df=1)	518*	637*	612*	265*	3517*	523*	2136*	2021*	363*	452*	159.4*	927.5*	464*	3710*
	Insecticide (df=3)	57.3*	80.2*	55.5*	28.5*	504*	117*	427*	306*	67*	54*	25.4*	95.2*	95.5*	657.4*
	Inoculation × insecticide (df=3)	35.4*	73.6*	33.7*	21.7*	220*	72.6*	283*	85*	31.2*	35.3*	9.3*	57*	53.9*	204*

[†]Strain PS1 at 200, 400 and 600 µg L^{-1} of fipronil, produced 37, 31 and 24 µg L^{-1} SA; 20, 14 and 9 µg L^{-1} DHBA; 18, 14 and 12 µg ml^{-1} IAA and 19, 24 and 26 µg ml^{-1} EPS, respectively

Table 80 Effect of three concentrations of pyriproxyfen on growth and symbiotic properties of greengram plants grown in soil inoculated with *Pseudomonas aeruginosa* strain PS1[†] and without bioinoculant

Treatment	Dose rate (µg/ kg soil)	Length/ plant (cm)				Dry biomass (g/ plant)				Nodulation				Total dry biomass (g /plant)	
		Root		Shoot		Root		Shoot		No./ plant		Dry biomass (mg/ plant)			
		50 DAS	80 DAS	50 DAS	80 DAS	50 DAS	80 DAS	50 DAS	80 DAS	50 DAS	80 DAS	50 DAS	80 DAS	50 DAS	80 DAS
Uninoculated	Control	28	30	14	16	0.35	0.47	1.59	2.08	21	17	66	52	2.00	2.60
	1300	16	17	10	12	0.19	0.26	0.96	1.33	18	15	54	43	1.12	1.63
	2600	15	16	8	10	0.16	0.22	0.83	1.24	14	12	47	37	1.04	1.49
	3900	11	12	7	9	0.10	0.19	0.76	1.06	12	8	44	32	1.00	1.28
Inoculated	Control	33	36	30	32	1.76	2.56	8.70	11.66	41	36	399	316	10.86	1.45
	1300	23	34	29	31	1.10	1.56	5.50	9.03	30	25	207	177	7.52	10.77
	2600	26	27	24	26	1.03	1.23	4.56	7.90	26	23	175	144	5.77	9.27
	3900	21	22	23	25	0.76	1.03	3.90	6.50	24	17	142	116	4.80	7.65
LSD		1.5	0.62	0.73	0.74	1.3	1.8	1.2	1.8	2.4	0.83	0.71	1.3	1.5	2.1
F value	Inoculation(df=1)	310*	249*	310*	273*	129*	107*	338*	507*	154.6*	1183*	94.5*	81.9*	622*	293.4*
	Insecticide (df=3)	42.3*	17*	8.6*	12*	29*	29.6*	62*	91*	49.0*	96*	25.4*	52.9*	62*	57.6*
	Inoculation × insecticide (df=3)	40.2*	17*	17.2*	11*	14*	8.2*	46*	65*	26.1*	68*	15.8*	15.3*	46*	47.0*

[†]Strain PS1 at 1300, 2600 and 3900 µg L^{-1} of pyriproxyfen, produced 36, 30 and 22 µg L^{-1} SA; 12, 8 and 6 µg L^{-1} DHBA; 15, 9 and 6 µg ml^{-1} IAA and 18, 25 and 27 µg ml^{-1} EPS, respectively

Table 81 Effect of three concentrations of tebuconazole on growth and symbiotic properties of greengram plants grown in soil inoculated with *Pseudomonas aeruginosa* strain PS1[†] and without bioinoculant

Treatment	Dose rate (µg/ kg soil)	Length/ plant (cm)				Dry biomass (mg/ plant)				Nodulation				Total dry biomass (g /plant)	
		Root		Shoot		Root		Shoot		No./ plant		Dry biomass (mg/ plant)			
		50 DAS	80 DAS	50 DAS	80 DAS	50 DAS	80 DAS	50 DAS	80 DAS	50 DAS	80 DAS	50 DAS	80 DAS	50 DAS	80 DAS
Uninoculated	Control	28	30	14	16	0.35	0.47	1.59	2.08	21	17	66	52	2.00	2.60
	100	22	24	13	14	0.29	0.37	1.18	1.34	16	13	50	44	1.52	1.76
	200	18	20	11	12	0.24	0.32	1.06	1.20	12	9	44	38	1.35	1.56
	300	18	19	9	10	0.23	0.27	0.95	1.08	10	8	40	32	1.22	1.38
Inoculated	Control	33	36	30	32	1.76	2.56	8.70	11.66	41	36	399	316	1.09	14.54
	100	30	33	27	29	1.03	1.50	6.86	8.53	28	25	152	125	8.04	10.16
	200	26	28	23	25	0.83	1.40	4.46	7.70	28	22	137	110	5.43	9.21
	300	23	24	19	20	0.73	1.10	3.53	6.86	27	16	128	83	4.39	8.04
LSD		1.9	2.3	2.5	2.7	3.5	3.3	6.4	6.2	0.7	1.8	0.8	1.4	7.9	8.5
F value	Inoculation(df=1)	551*	408*	147*	505*	2288*	451*	1028*	2460*	252*	1027*	93.4*	258*	2824*	364*
	Pesticides (df=3)	70.4*	45.7*	14.7*	43.5*	421*	95.2*	272*	423*	28*	63*	7.7*	29.0*	660*	79.5*
	Interaction (df=3)	61.7*	27.9*	13*	25.7*	502*	86.3*	276*	392*	18.6*	73*	6.6*	20.8*	488*	81.9*

[†]Strain PS1 at 100, 200 and 300 µg L^{-1} of tebuconazole, produced 27, 23 and 20 µg L^{-1} SA; 8, 7 and 5 µg L^{-1} DHBA; 9, 5 and 3 µg ml^{-1} IAA and 19, 20 and 23 µg ml^{-1} EPS, respectively

Table 82 Effect of three concentrations of quizalafop-p-ethyl on biological and chemical properties of greengram plants grown in soil inoculated with *Pseudomonas aeruginosa* strain PS1 and without bioinoculant

Treatment	Dose rate (µg/ kg soil)	Leghaemoglobin content [mM (g f.m.)$^{-1}$]	Chlorophyll content (mg/g)	N content (mg/g)		P content (mg/g)		Seed yield (g/plant)	Seed protein (mg/g)
				Root	Shoot	Root	Shoot		
Uninoculated	Control	0.08	0.82	36	50	0.27	0.36	7.4	261
	40	0.06	0.74	27	38	0.18	0.28	3.6	235
	80	0.04	0.72	25	32	0.16	0.25	3.2	233
	120	0.03	0.68	20	28	0.13	0.23	2.8	230
Inoculated	Control	0.09	0.96	48	69	0.35	0.48	12.7	272
	40	0.07	0.78	33	52	0.28	0.37	8.2	253
	80	0.06	0.76	29	47	0.25	0.35	7.6	251
	120	0.04	0.75	27	42	0.23	0.33	7.2	248
LSD		0.008	0.04	1.4	2.6	0.002	0.003	0.23	2.1
F value	Inoculation (df=1)	609*	29.6*	242.4*	165.5*	512.2*	982*	207.3*	27*
	Herbicide (df=3)	2.3	1.5	110.2*	40.1*	109.1*	204.2*	41.7*	18.4*
	Inoculation × herbicide (df=3)	87.5*	1.8	40.1*	19.5*	27*	35.5*	22.3*	2.8

Table 83 Effect of three concentrations of clodinafop on biological and chemical properties of greengram plants grown in soil inoculated with *Pseudomonas aeruginosa* strain PS1 and without bioinoculant

Treatment	Dose rate (µg/ kg soil)	Leghaemoglobin content [mM (g f.m.)$^{-1}$]	Chlorophyll content (mg/g)	N content (mg/g)		P content (mg/g)		Seed yield (g/plant)	Seed protein (mg/g)
				Root	Shoot	Root	Shoot		
Uninoculated	Control	0.08	0.82	36	50	0.27	0.36	7.4	261
	400	0.07	0.80	35	48	0.26	0.34	6.4	258
	800	0.06	0.78	32	45	0.25	0.31	6.1	255
	1200	0.05	0.75	30	42	0.23	0.29	5.3	251
Inoculated	Control	0.09	0.96	48	69	0.35	0.48	12.7	272
	400	0.08	0.93	45	65	0.32	0.45	11.5	269
	800	0.08	0.87	41	61	0.29	0.42	10.4	264
	1200	0.07	0.85	38	58	0.26	0.39	9.7	258
LSD		0.006	0.03	1.2	1.8	0.003	0.003	0.04	1.8
F value	Inoculation (df= 1)	532.5*	197.2*	41.3*	71.3*	217.3*	67.9*	168.5*	68.4*
	Herbicide (df=3)	63.2*	7.2*	6.3*	4.3*	84.3*	5.3*	54.3*	46.3*
	Inoculation × herbicide (df=3)	47.2*	2.4	2.1	1.6	21.5*	2.4	33.2*	2.8

Table 84 Effect of three concentrations of fipronil on biological and chemical properties of greengram plants grown in soil inoculated with *Pseudomonas aeruginosa* strain PS1 and without bioinoculant

Treatment	Dose rate (µg/ kg soil)	Leghaemoglobin content [mM (g f.m.)$^{-1}$]	Chlorophyll content (mg/g)	N content (mg/g)		P content (mg/g)		Seed yield (g/plant)	Seed protein (mg/g)
				Root	Shoot	Root	Shoot		
Uninoculated	Control	0.08	0.82	36	50	0.27	0.36	7.4	261
	200	0.07	0.77	33	44	0.25	0.35	6.2	256
	400	0.06	0.74	29	42	0.23	0.33	5.7	253
	600	0.05	0.72	24	39	0.21	0.31	4.6	248
Inoculated	Control	0.09	0.96	48	69	0.35	0.48	12.7	272
	200	0.08	0.89	43	63	0.31	0.43	10.5	268
	400	0.07	0.87	41	59	0.29	0.40	9.7	266
	600	0.06	0.85	37	55	0.27	0.37	8.5	264
LSD		0.016	0.16	1.3	1.5	0.04	0.06	0.08	6.3
F value	Inoculation (df= 1)	523.5*	1719.6*	28.3*	235.4*	192.1*	44.1*	1126*	106.5*
	Insecticide (df=3)	44.5*	163.5*	3.1	13.5*	53.4*	2.9	119*	16.4*
	Inoculation × insecticide (df=3)	31.4*	103.2*	1.5	1.4	13.7*	2.5	41.8*	12.3*

Table 85 Effect of three concentrations of pyriproxyfen on biological and chemical properties of greengram plants grown in soil inoculated with *Pseudomonas aeruginosa* strain PS1 and without bioinoculant

Treatment	Dose rate (µg/ kg soil)	Leghaemoglobin content [mM (g f.m.)$^{-1}$]	Chlorophyll content (mg/g)	N content (mg/g)		P content (mg/g)		Seed yield (g/plant)	Seed protein (mg/g)
				Root	Shoot	Root	Shoot		
Uninoculated	Control	0.08	0.82	36	50	0.27	0.36	7.4	261
	1300	0.07	0.76	31	43	0.22	0.31	5.5	251
	2600	0.06	0.74	27	37	0.19	0.29	5.1	247
	3900	0.04	0.71	23	34	0.17	0.27	4.5	244
Inoculated	Control	0.09	0.96	48	69	0.35	0.48	12.7	272
	1300	0.08	0.88	39	62	0.29	0.41	9.8	259
	2600	0.07	0.85	37	57	0.26	0.38	8.6	256
	3900	0.06	0.81	34	55	0.23	0.35	8.3	251
LSD		0.021	0.19	1.13	1.21	0.06	0.04	0.02	3.2
F value	Inoculation (df= 1)	301*	328.6*	114.3*	178.3*	21.1*	118.1*	677.4*	57.2*
	Insecticide (df=3)	17.4*	41.4*	11.6*	12.9*	9.5*	8.8*	71.2*	6.0*
	Inoculation × insecticide (df=3)	12.5*	27.2*	2.5	9.7*	1.6	9.5*	43.8*	3.2

Table 86 Effect of three concentrations of tebuconazole on biological and chemical properties of greengram plants grown in soil inoculated with *Pseudomonas aeruginosa* strain PS1 and without bioinoculant

Treatment	Dose rate (µg/ kg soil)	Leghaemoglobin content [mM (g f.m.)$^{-1}$]	Chlorophyll content (mg/g)	N content (mg/g)		P content (mg/g)		Seed yield (g/plant)	Seed protein (mg/g)
				Root	Shoot	Root	Shoot		
Uninoculated	Control	0.08	0.82	36	50	0.27	0.36	7.4	261
	100	0.07	0.75	29	41	0.21	0.29	4.8	246
	200	0.05	0.72	25	37	0.18	0.26	4.3	244
	300	0.04	0.70	27	35	0.17	0.24	3.8	241
Inoculated	Control	0.09	0.96	48	69	0.35	0.48	12.7	272
	100	0.08	0.84	36	56	0.30	0.39	9.2	257
	200	0.06	0.80	31	51	0.26	0.37	8.5	250
	300	0.05	0.75	29	45	0.21	0.35	7.8	246
LSD		0.005	0.04	1.5	2.4	0.05	0.02	0.3	2.5
F value	Inoculation (df= 1)	326*	7.2*	363*	47.3*	771.7*	1155*	265.5*	19*
	Fungicides (df=3)	8.7*	1.1	201.9*	35.1*	347.7*	74.7*	32.7*	11*
	Inoculation × fungicide (df=3)	31.5*	0.4	50.1*	14.5*	76.4*	119.5*	20.9*	3.5*

Table 87 Comparative analysis of pesticidal impact on number of nodules, seed yield and grain protein of uninoculated legumes grown in sandy clay loam soil treated with three times (3X) more of recommended rate of each

Pesticides	Three times of recommended dose rate (µg/ kg soil)	Crop	Percent decrease in measured parameters*		
			†Nodules no.	Seed yield	Seed protein
Quizalafop-p-ethyl	120	Chickpea	67	78	33
		Pea	100	50	4
		Greengram	48	63	12
		Lentil	100	80	7
Clodinafop	1200	Chickpea	24	38	7
		Pea	60	14	2
		Greengram	24	29	4
		Lentil	37	34	5
Fipronil	600	Chickpea	34	52	9
		Pea	67	15	2
		Greengram	43	38	5
		Lentil	37	47	6
Pyriproxyfen	3900	Chickpea	15	60	18
		Pea	63	15	2
		Greengram	43	40	7
		Lentil	43	57	7
Tebuconazole	300	Chickpea	48	67	24
		Pea	100	23	3
		Greengram	53	49	8
		Lentil	100	70	7

pesticide

*Values indicate percent decrease over control, †50 (for greengram) and 90 (for chickpea, pea and lentil) days after sowing

Figures

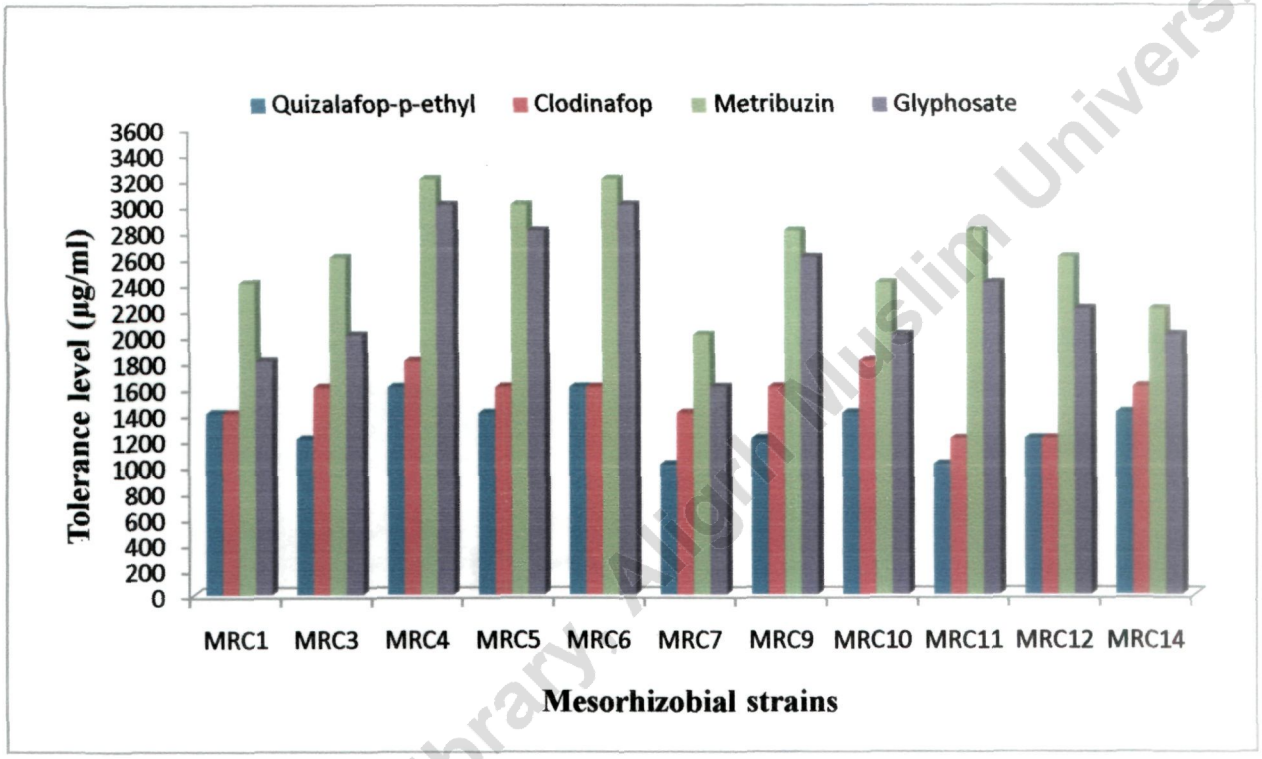


Fig. 13: Tolerance level of *Mesorhizobium* stains grown in minimal salt agar medium to herbicides

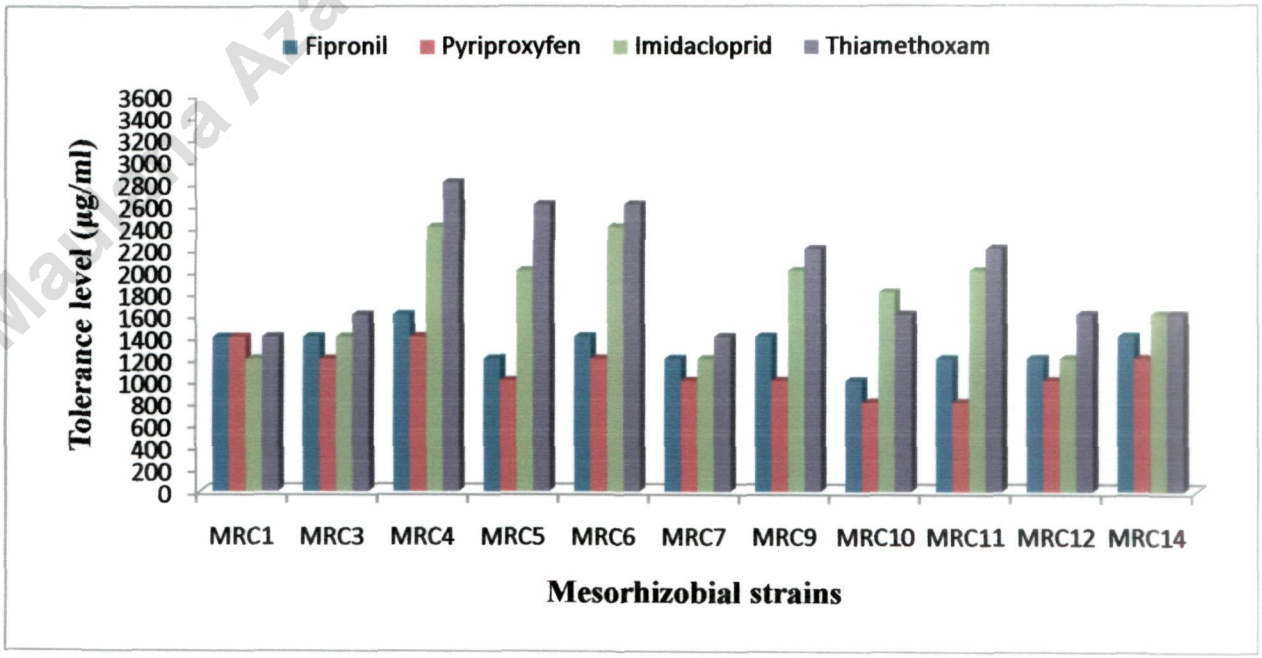


Fig. 14: Tolerance level of *Mesorhizobium* stains grown in minimal salt agar medium to insecticides

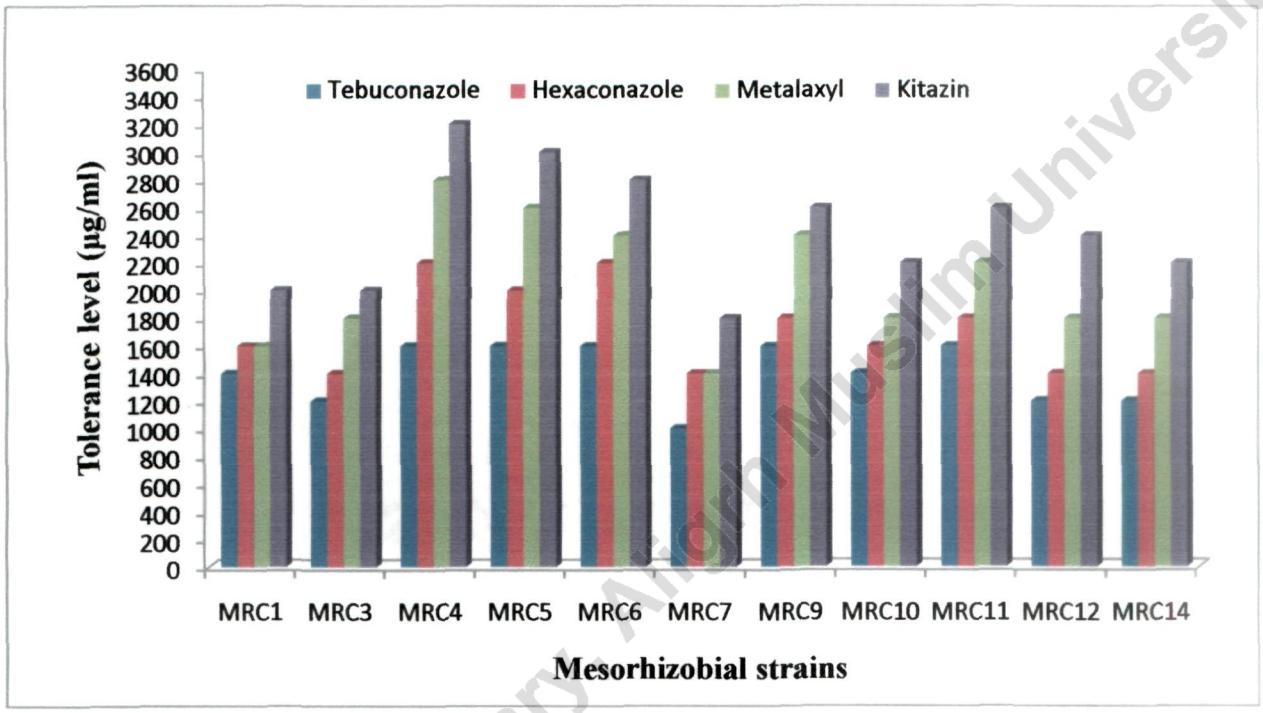


Fig. 15: Tolerance level of *Mesorhizobium* stains grown in minimal salt agar medium to fungicides

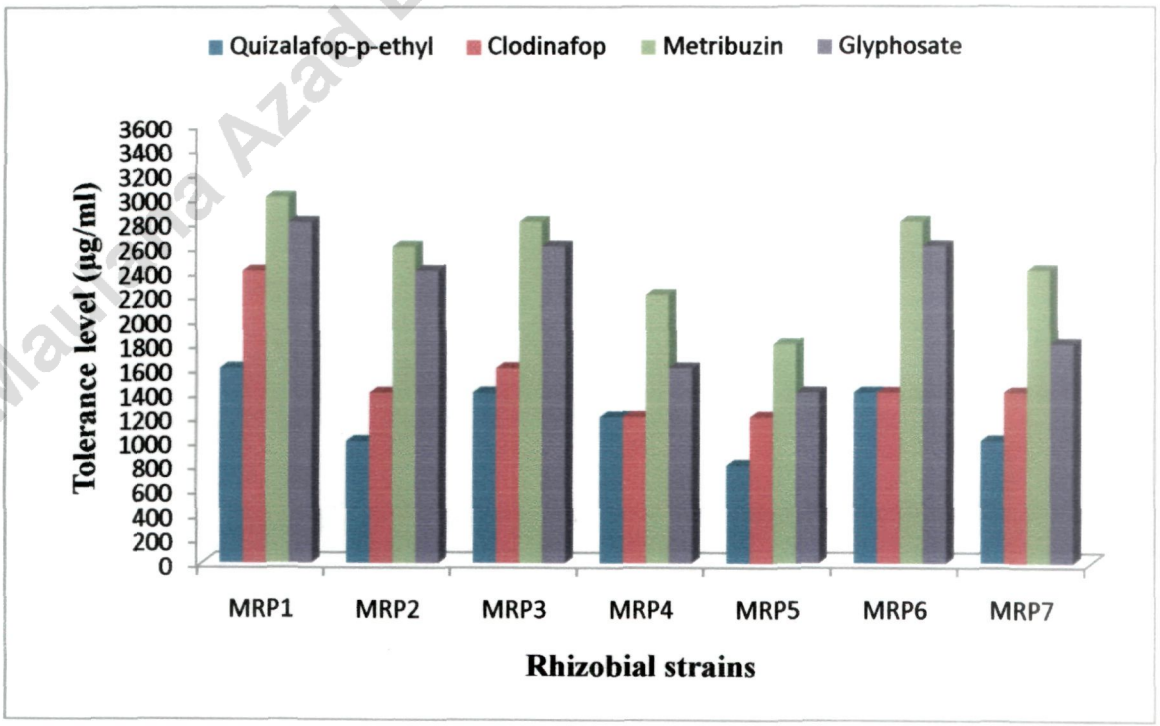


Fig. 16: Tolerance level of *Rhizobium* stains grown in minimal salt agar medium to herbicides

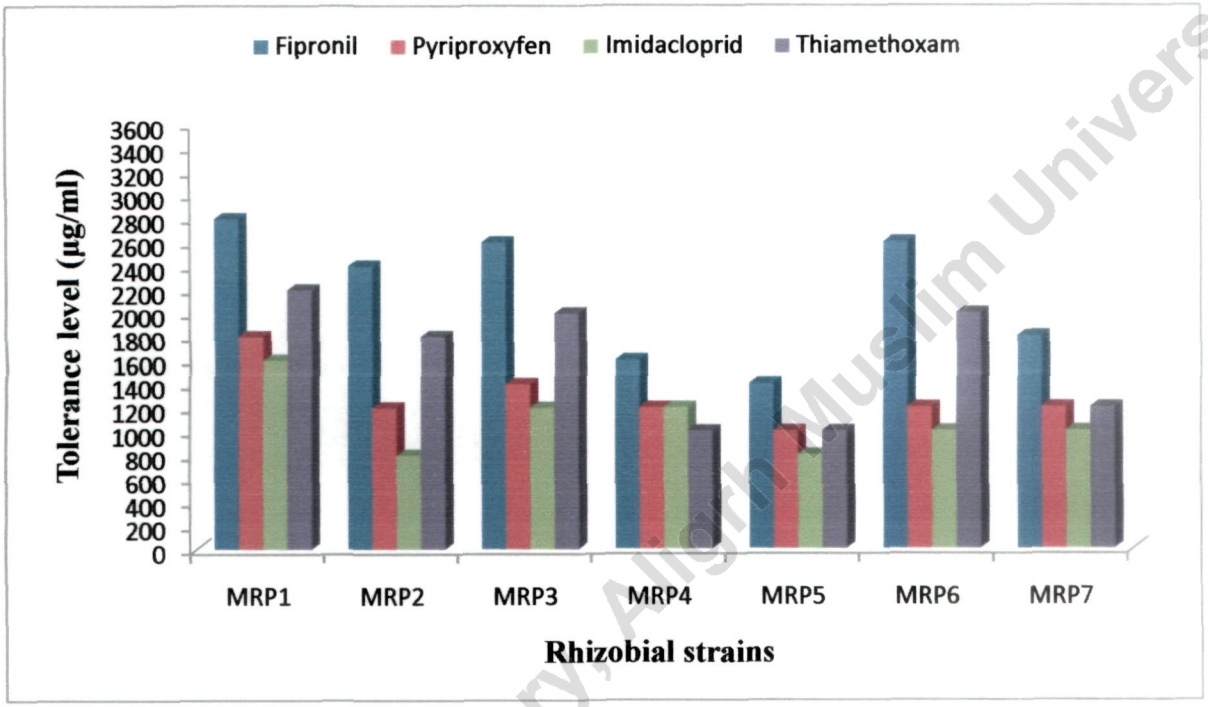


Fig. 17: Tolerance level of *Rhizobium* strains grown in minimal salt agar medium to insecticides

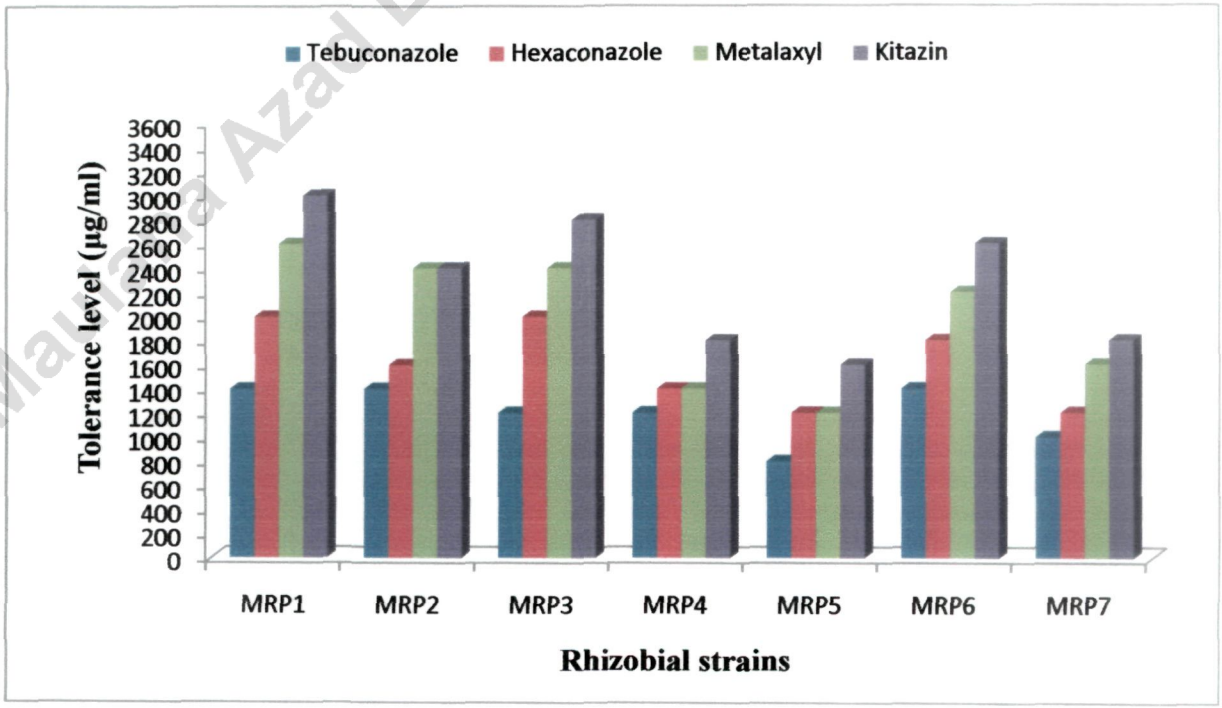


Fig. 18: Tolerance level of *Rhizobium* strains grown in minimal salt agar medium to fungicides

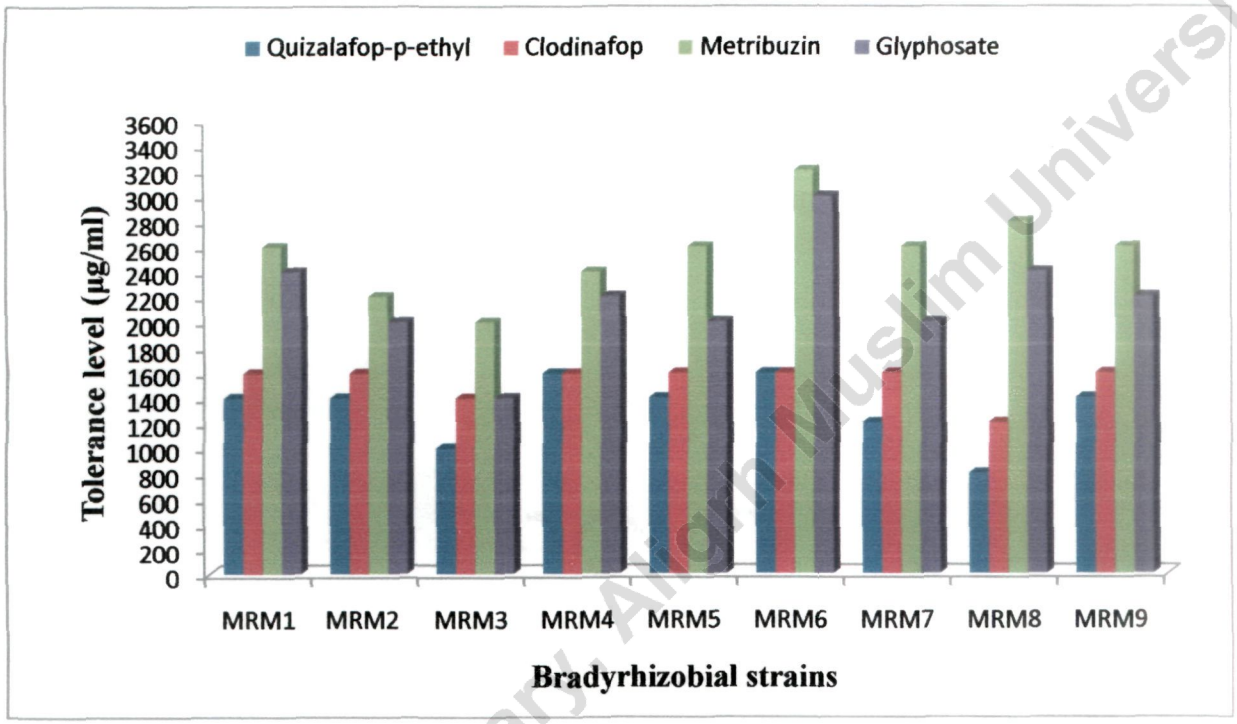


Fig. 19: Tolerance level of *Bradyrhizobium* stains grown in minimal salt agar medium to herbicides

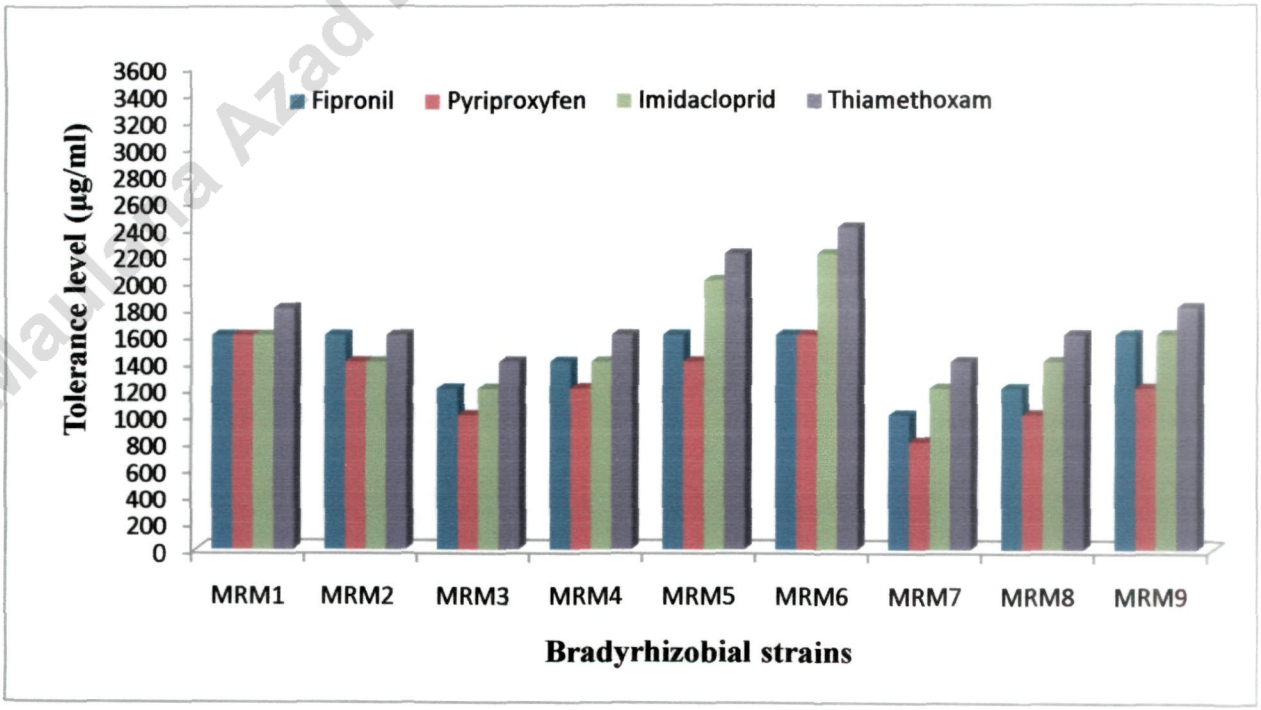


Fig. 20: Tolerance level of *Bradyrhizobium* stains grown in minimal salt agar medium to insecticides

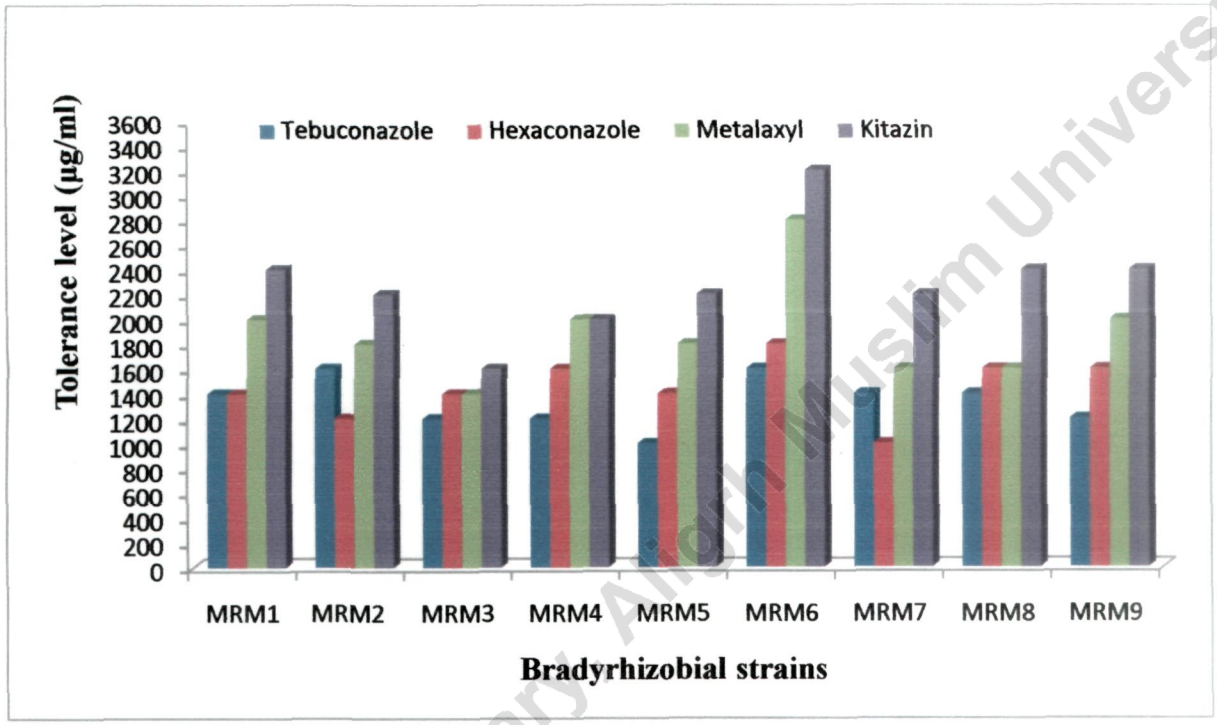


Fig. 21: Tolerance level of *Bradyrhizobium* stains grown in minimal salt agar medium to herbicides

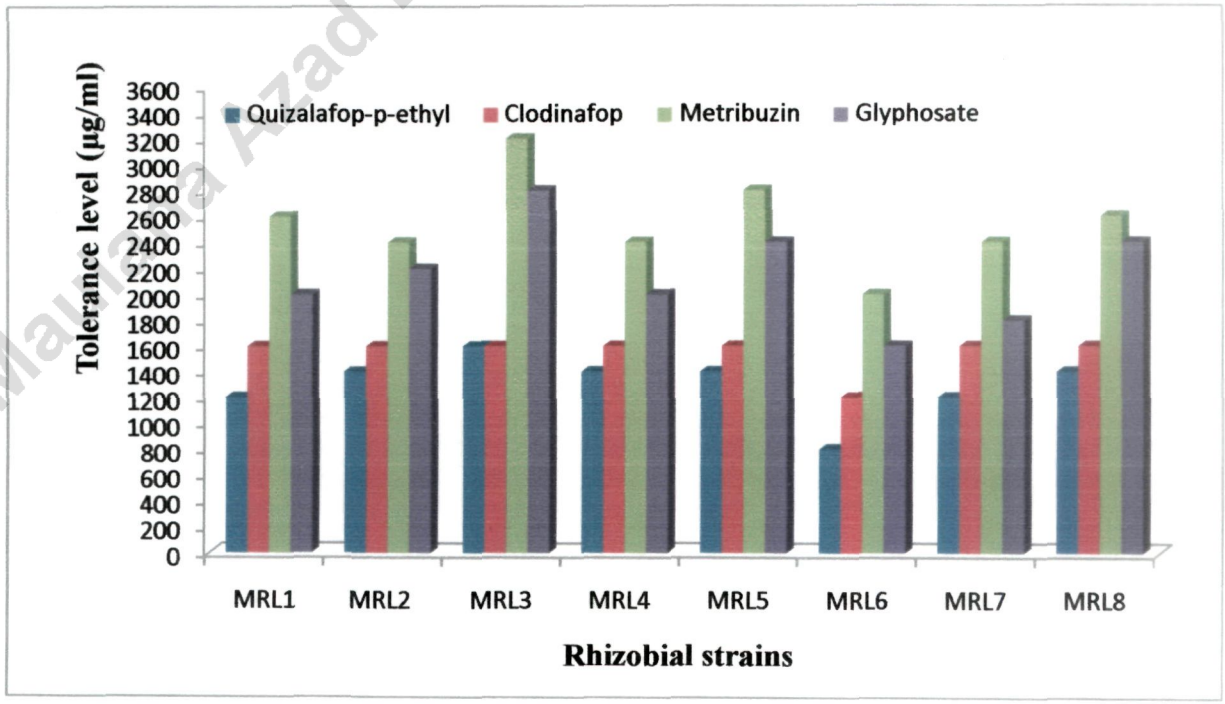


Fig. 22: Tolerance of *Rhizobium* stains grown in minimal salt agar medium to herbicides

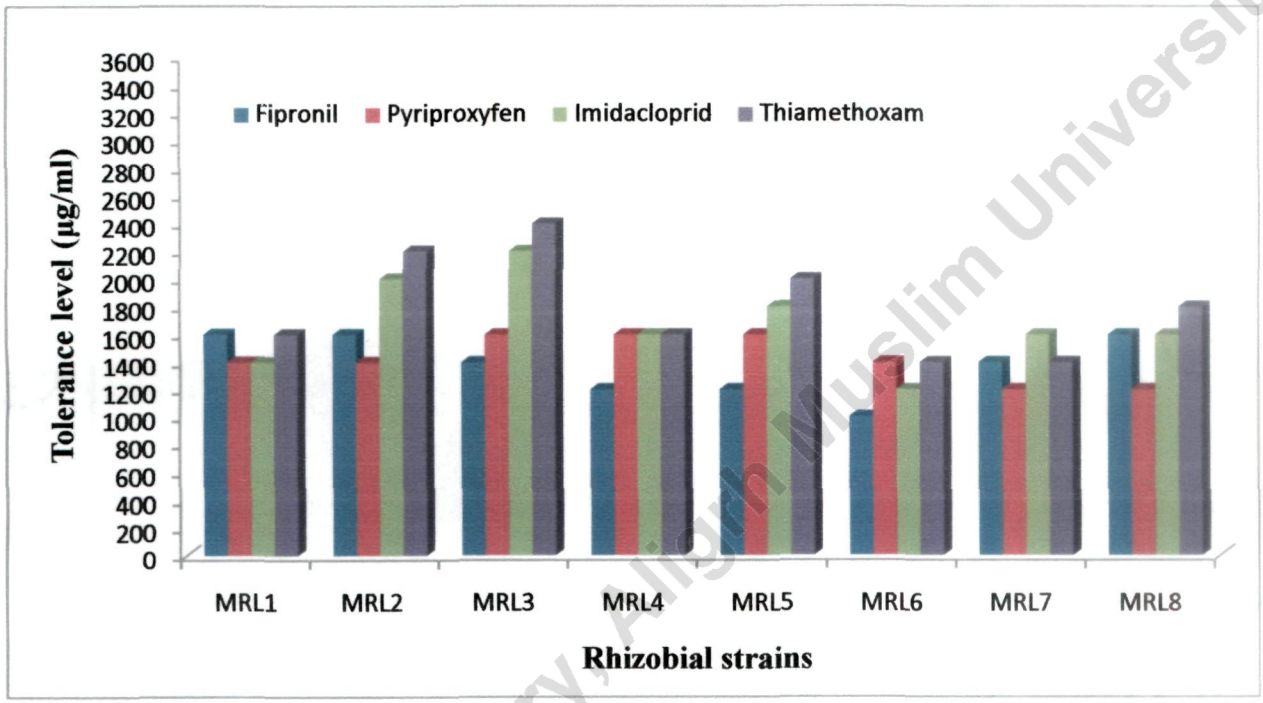


Fig. 23: Tolerance of *Rhizobium* stains grown in minimal salt agar medium to insecticides

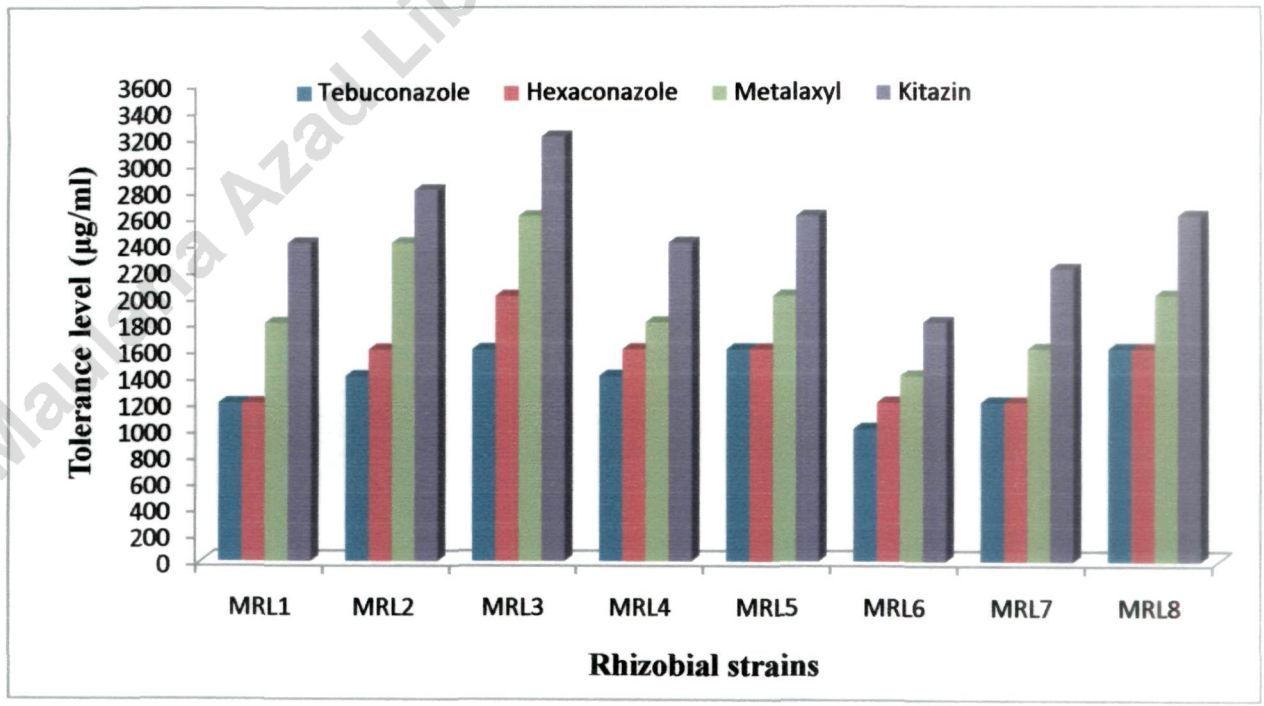


Fig. 24: Tolerance level of *Rhizobium* stains grown in minimal salt agar medium to fungicides

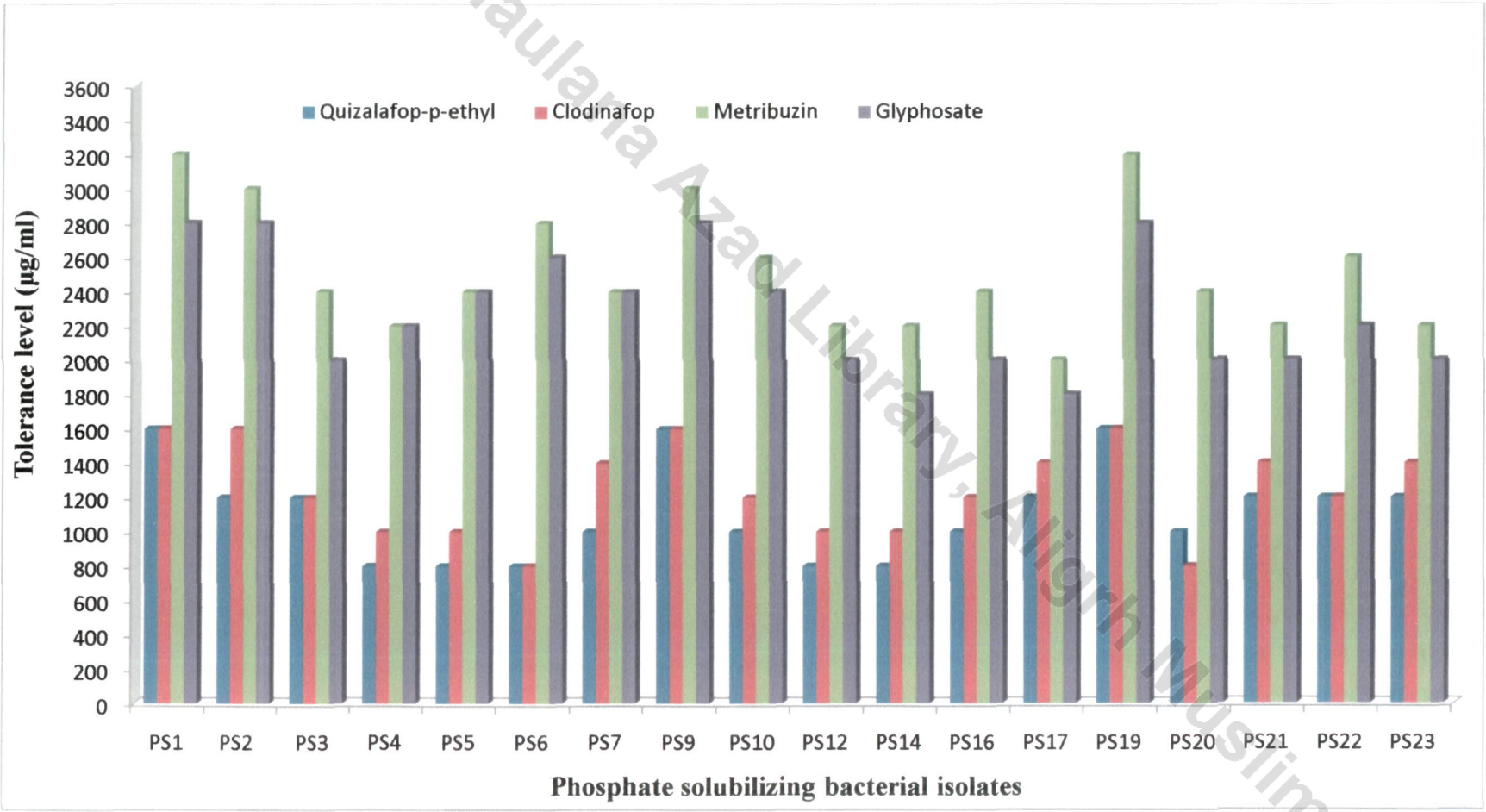


Fig. 25: Tolerance level of to phosphate solubilizing bacterial stains grown in minimal salt agar medium to herbicides

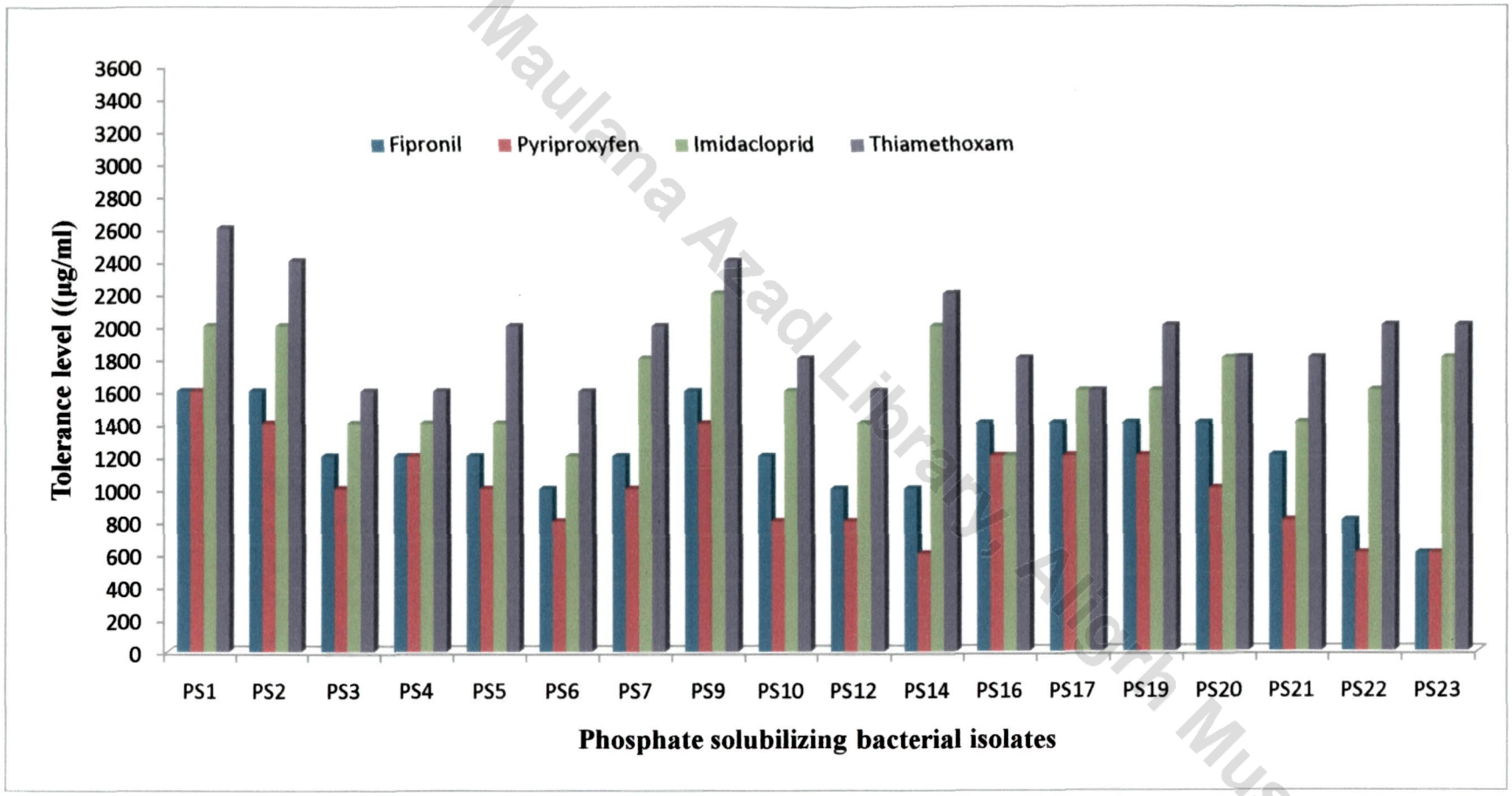


Fig. 26: Tolerance level of phosphate solubilizing bacterial stains grown in minimal salt agar medium to insecticides

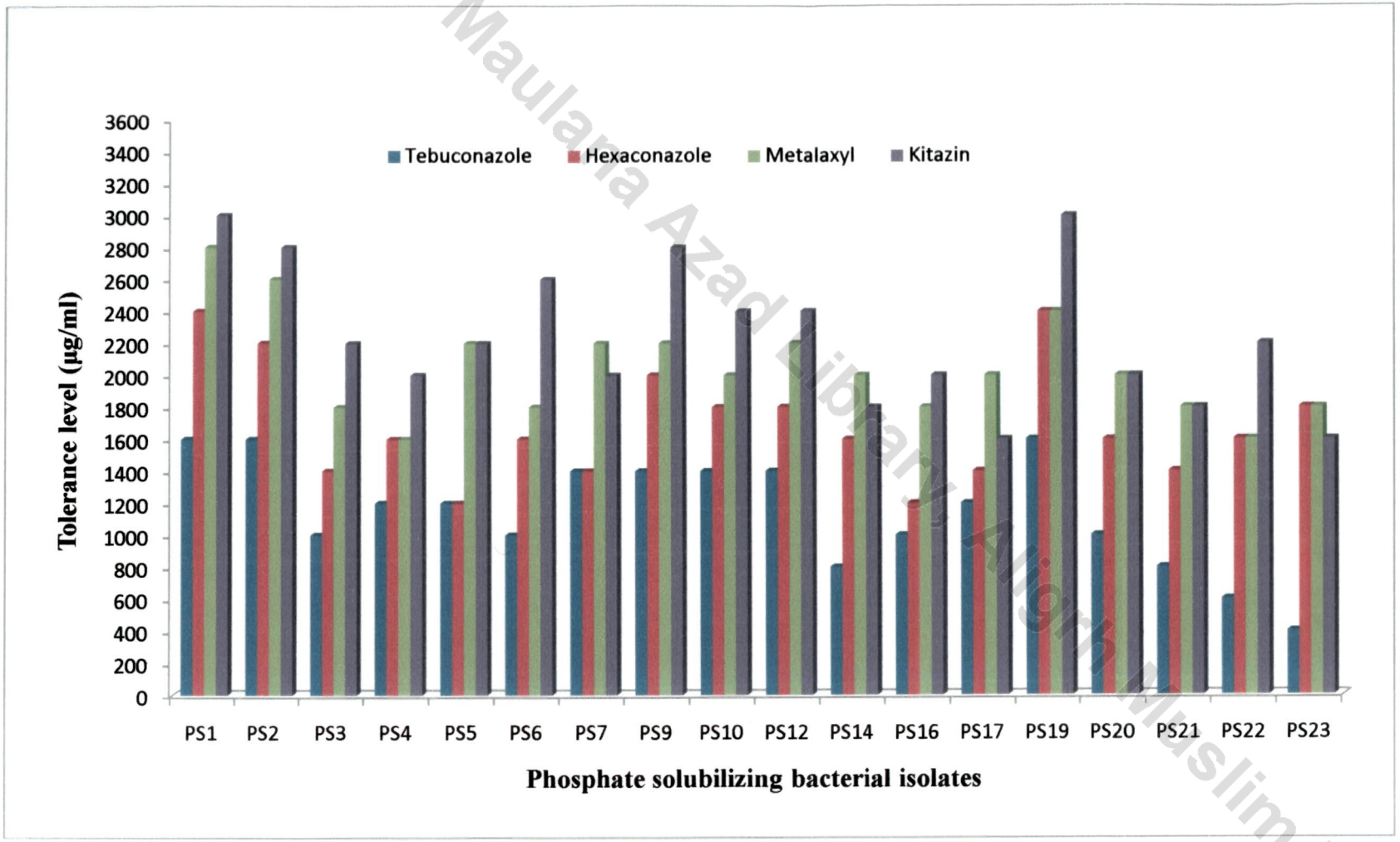


Fig. 27: Tolerance level of phosphate solubilizing bacterial stains grown in minimal salt agar medium to fungicides

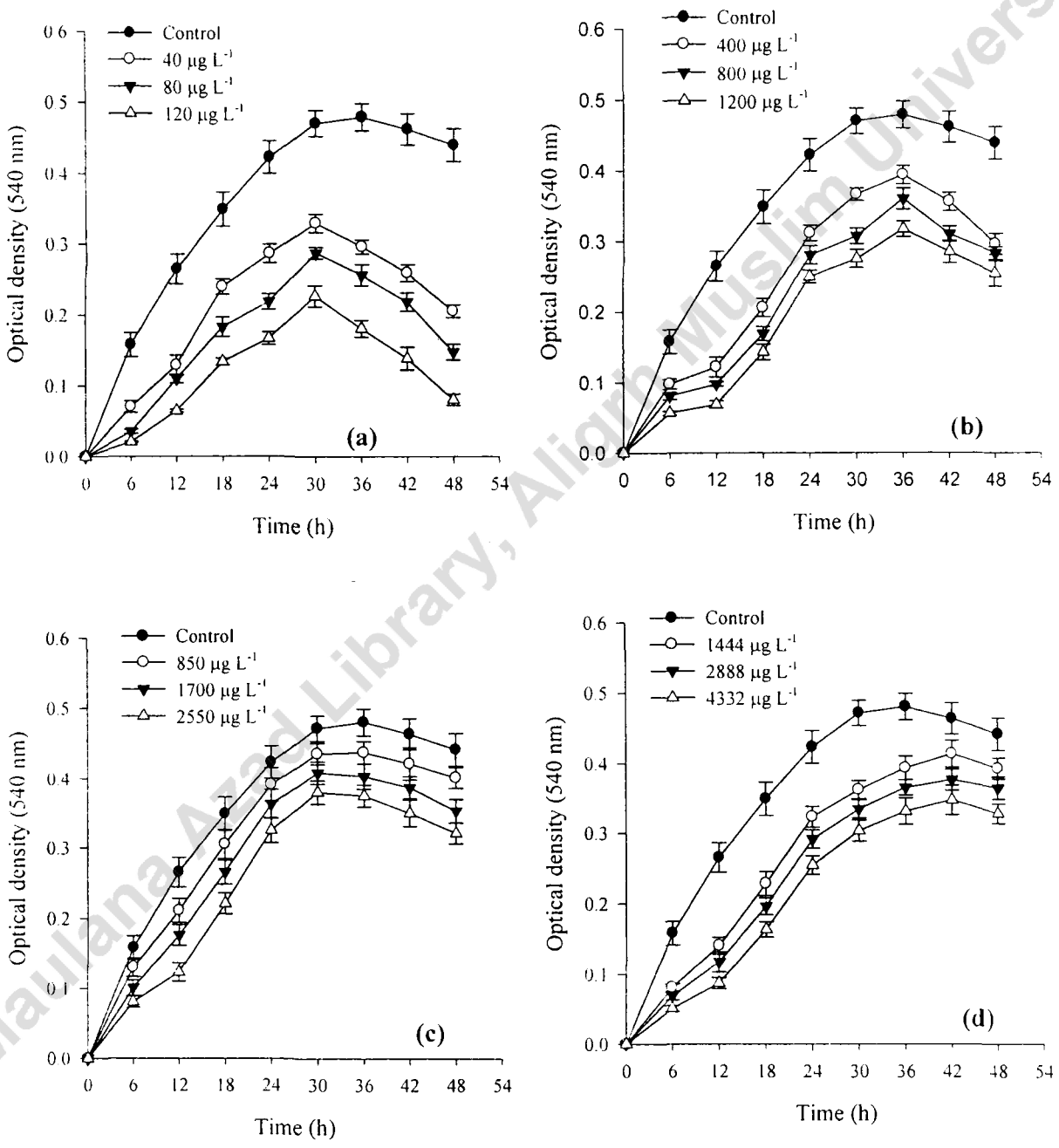


Fig. 28: Impact of recommended (○), double (▼) and three times more (Δ) of recommended rates of quizalafop-p-ethyl (a), clodinafop (b), metribuzin (c) and glyphosate (d) on *Mesorhizobium* strain MRC4 (in terms of optical density) grown in minimal salt agar medium

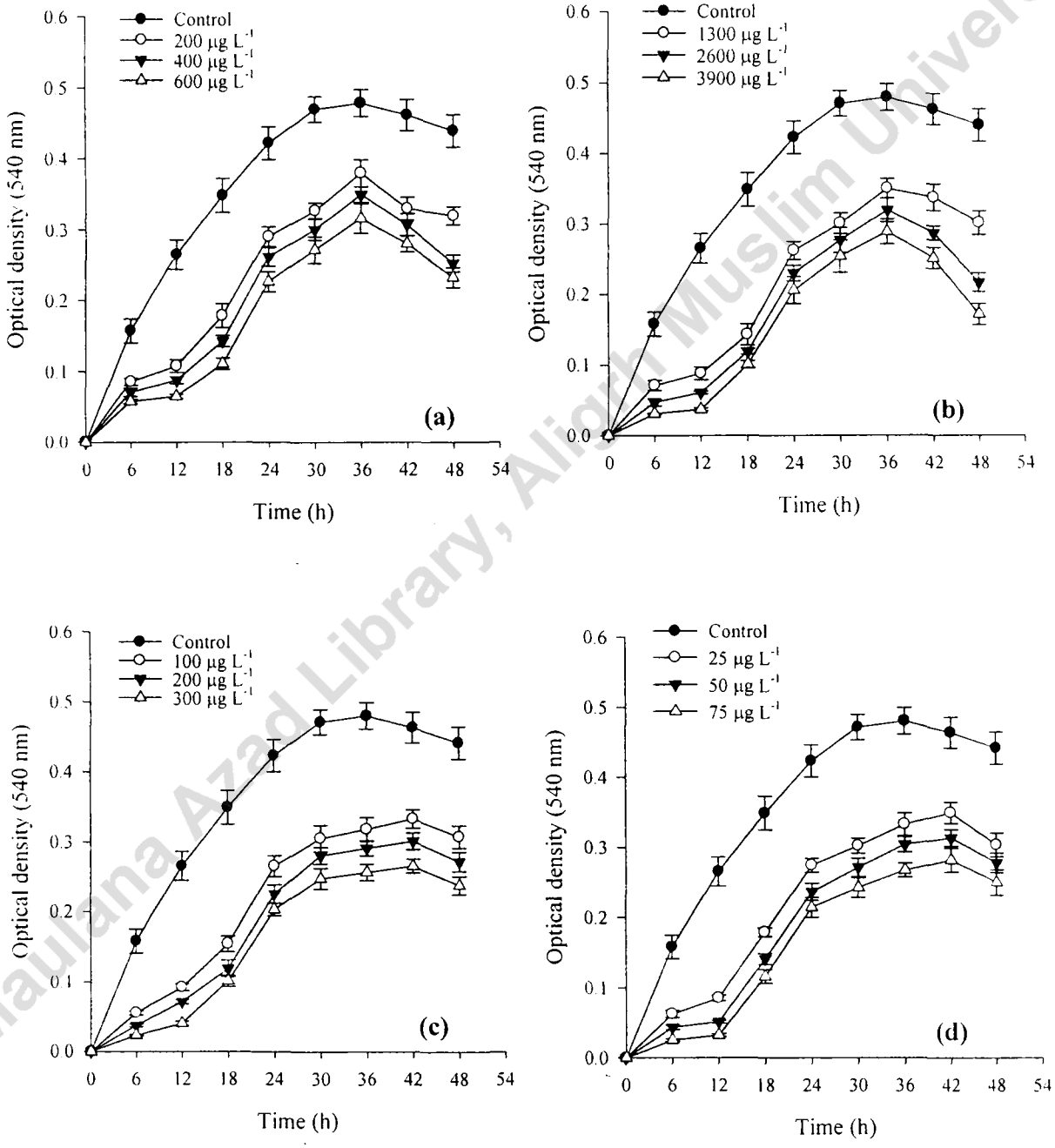


Fig. 29: Impact of recommended (○), double (▼) and three times more (Δ) of recommended rates of fipronil (a), pyriproxyfen (b), imidacloprid (c) and thiamethoxam (d) on *Mesorhizobium* strain MRC4 (in terms of optical density) grown in minimal salt agar medium

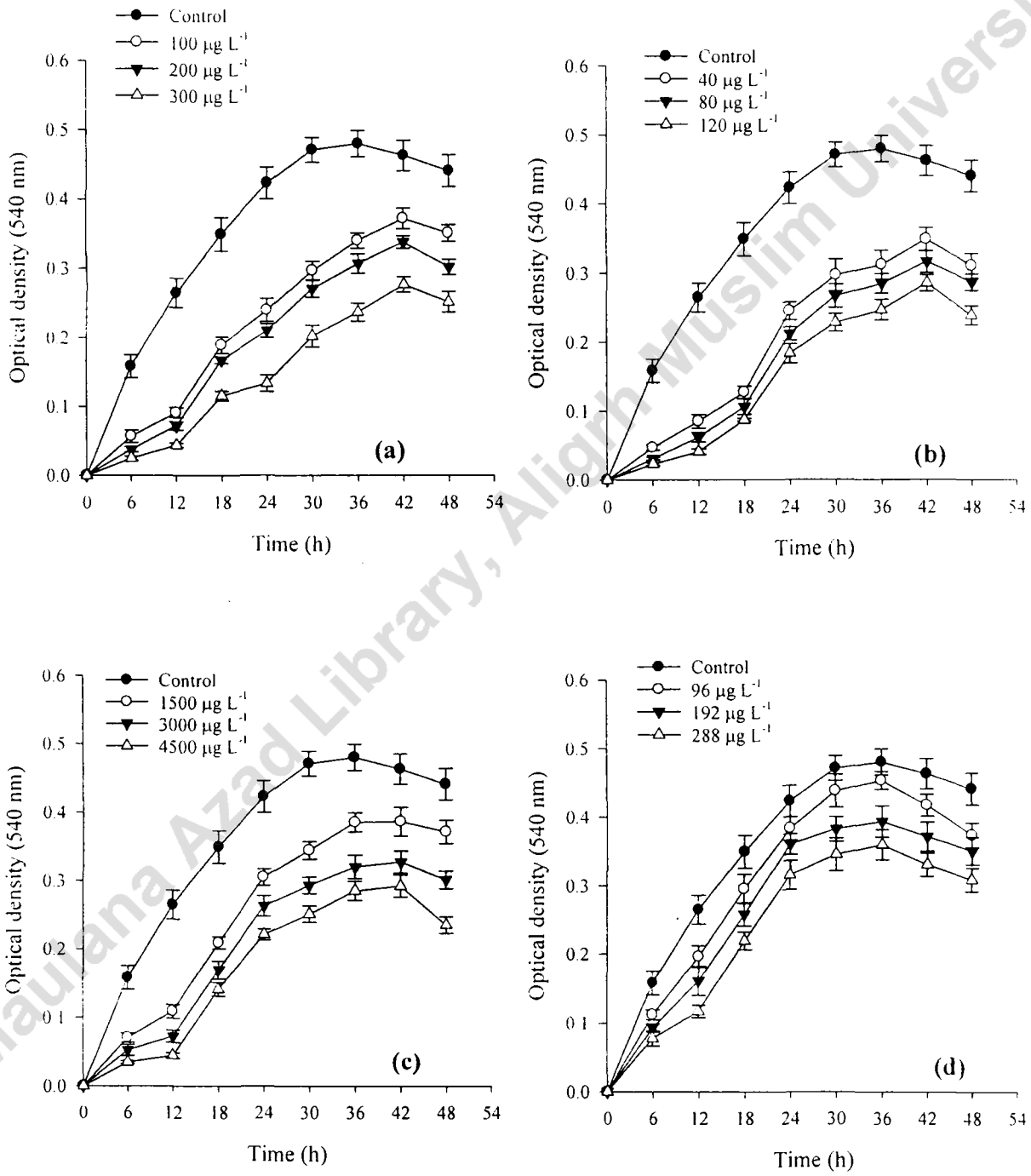


Fig. 30: Impact of recommended (○), double (▼) and three times more (Δ) of recommended rates of tebuconazole (a), hexaconazole (b), metalaxyl (c) and kitazin (d) on *Mesorhizobium* strain MRC4 (in terms of optical density) grown in minimal salt agar medium

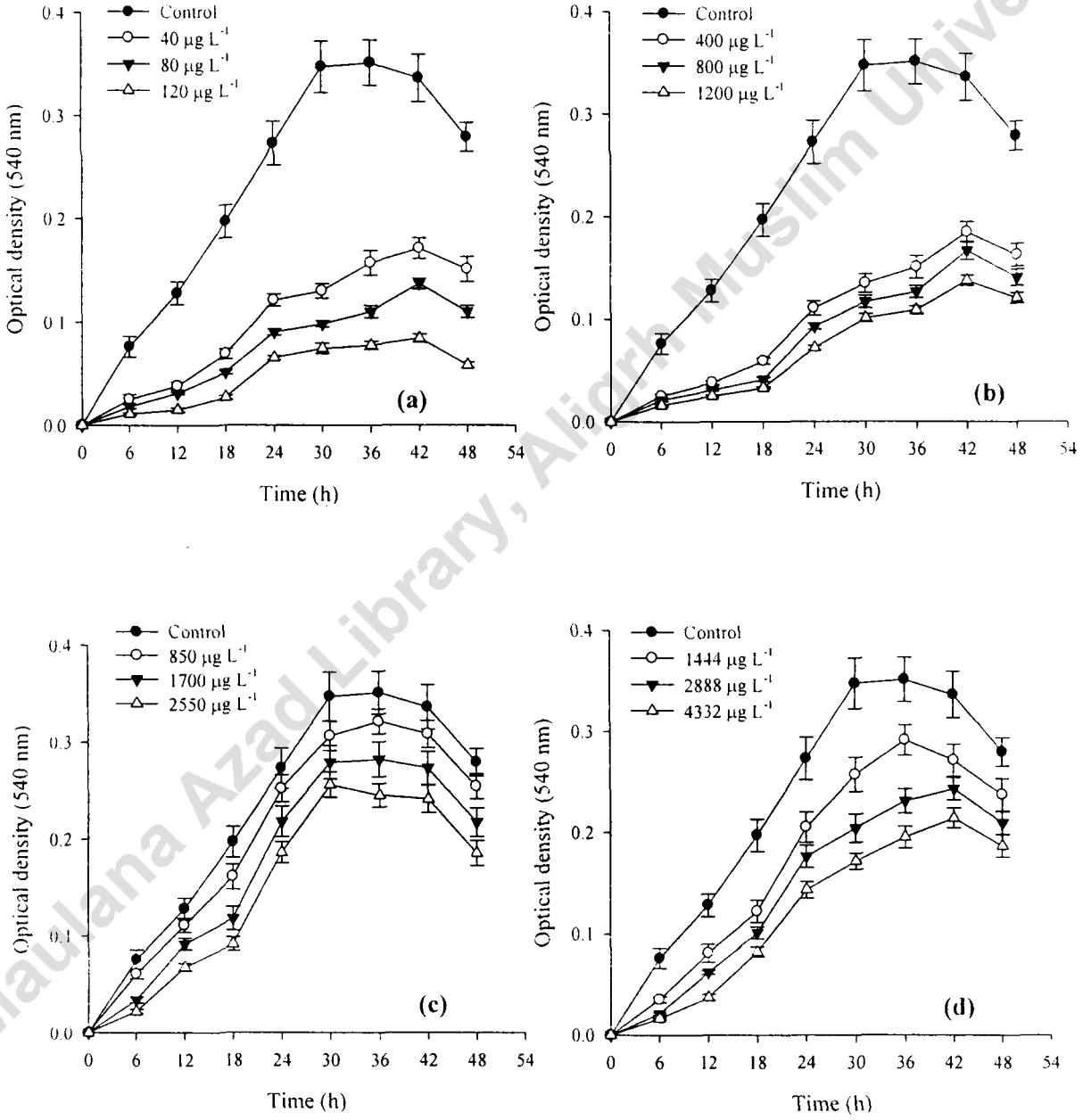


Fig. 31: Impact of recommended (○), double (▼) and three times more (Δ) of recommended rates of quizalafop-p-ethyl (a), clodinafop (b), metribuzin (c) and glyphosate (d) on *Rhizobium* strain MRP1 (in terms of optical density) grown in minimal salt agar medium

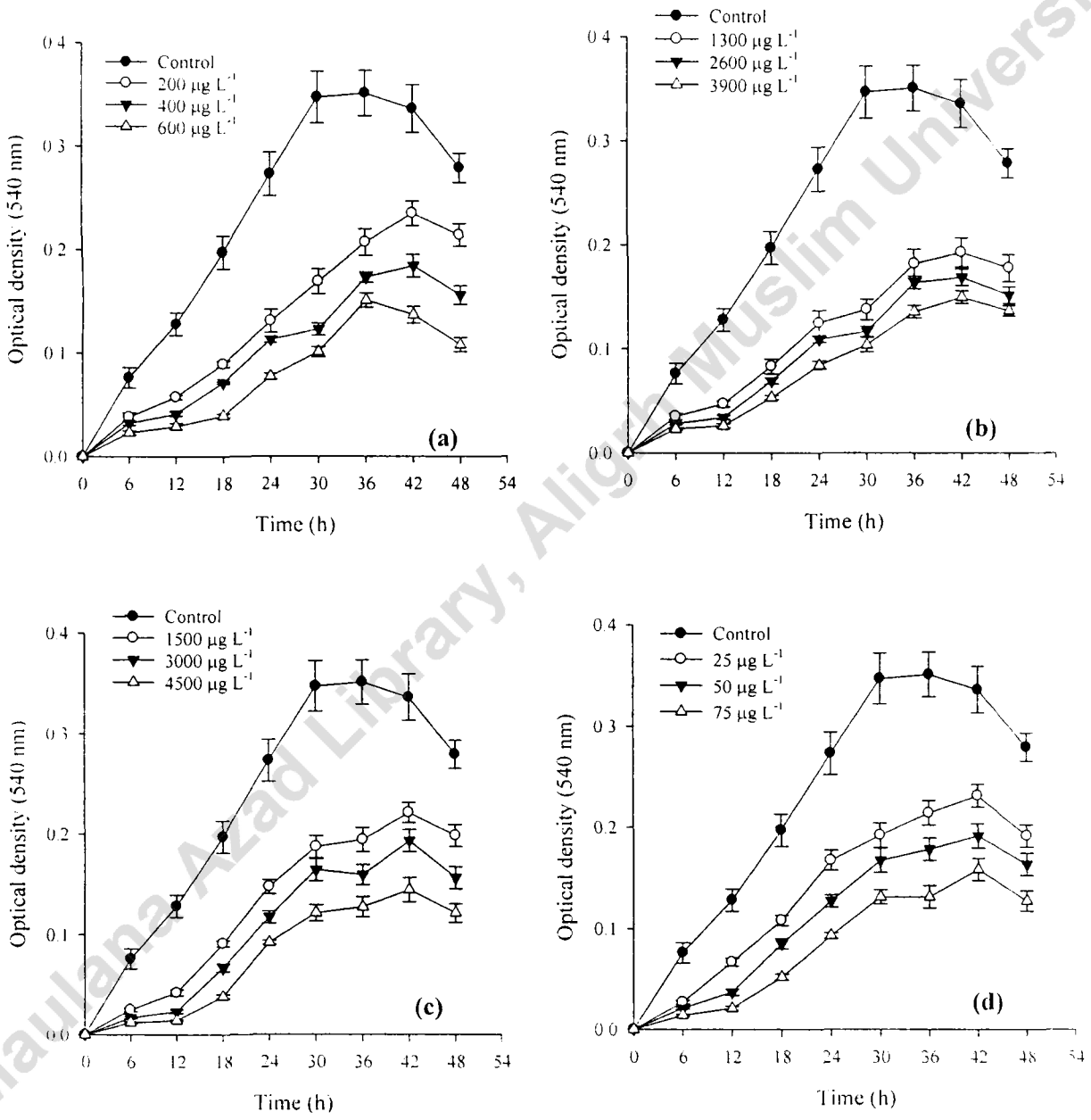


Fig. 32 Impact of recommended (○), double (▼) and three times more (Δ) of recommended rates of fipronil (a), pyriproxyfen (b), imidacloprid (c) and thiamethoxam (d) on *Rhizobium* strain MRP1 (in terms of optical density) grown in minimal salt agar medium

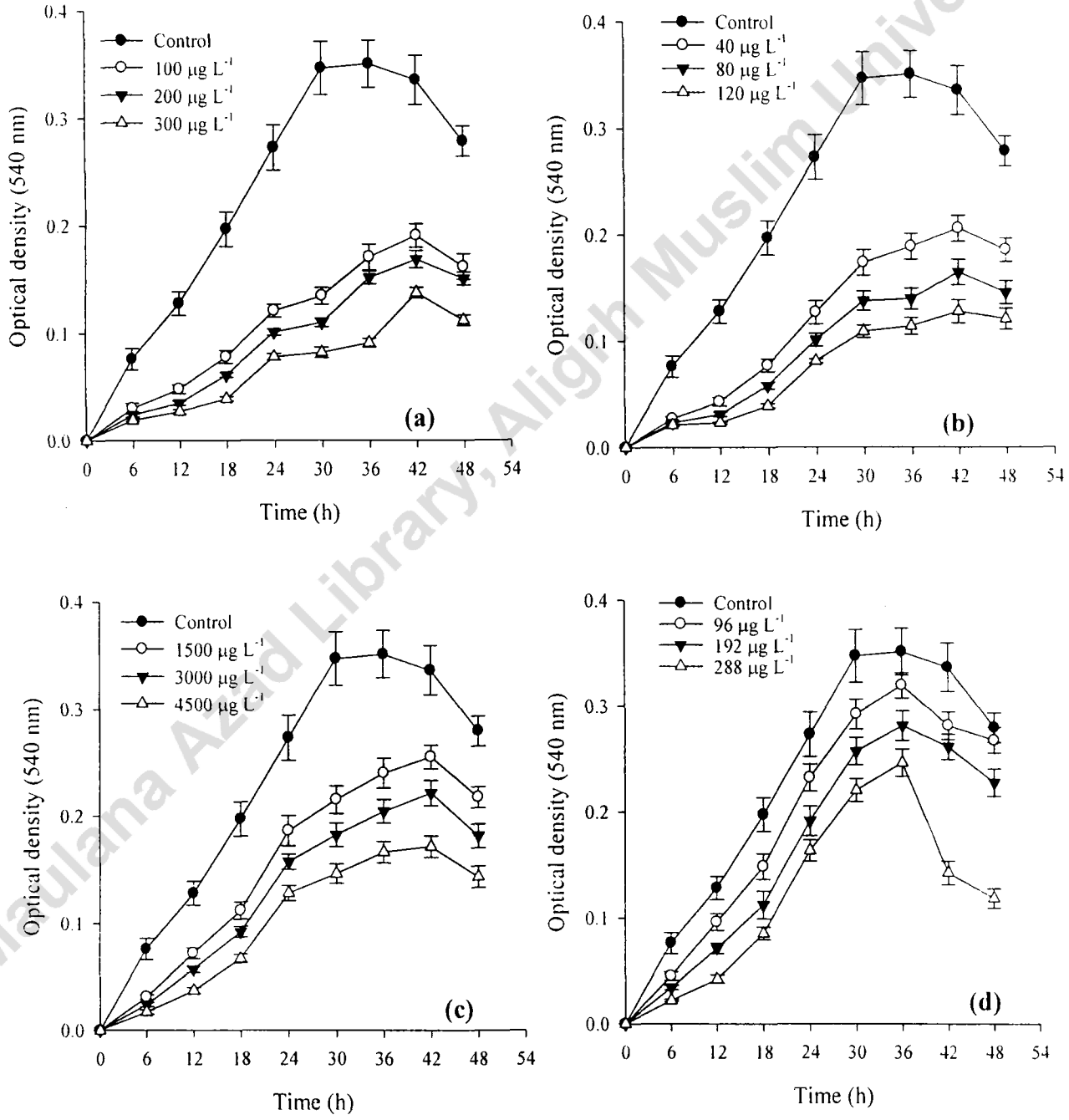


Fig. 33: Impact of recommended (○), double (▼) and three times more (Δ) of recommended rates of tebuconazole (a), hexaconazole (b), metalaxyl (c) and kitazin (d) on *Rhizobium* strain MRP1 (in terms of optical density) grown in minimal salt agar medium

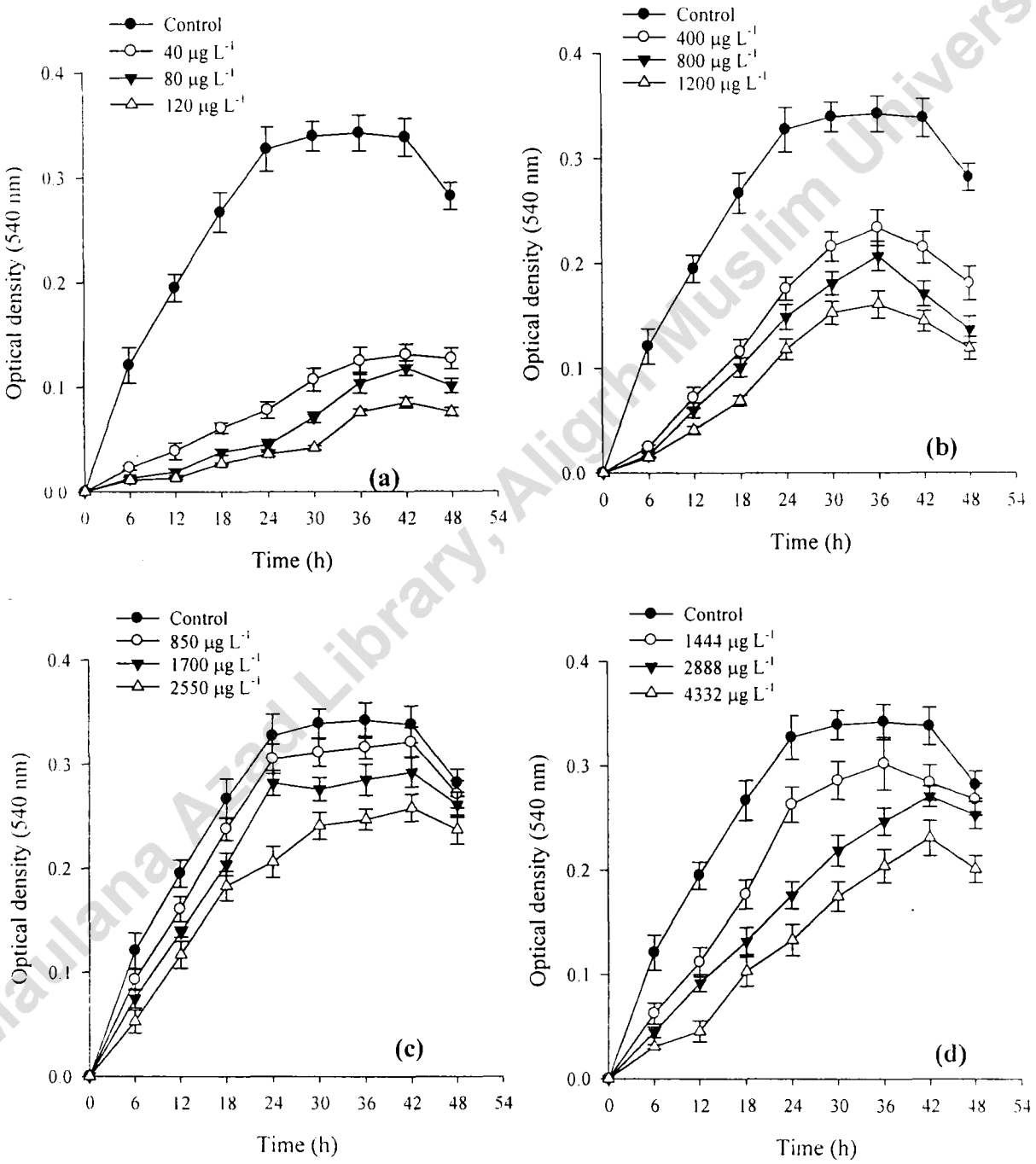


Fig. 34: Impact of recommended (○), double (▼) and three times more (Δ) of recommended rates of quizalafop-p-ethyl (a), clodinafop (b), metribuzin (c) and glyphosate (d) on *Bradyrhizobium* strain MRM6 (in terms of optical density) grown in minimal salt agar medium

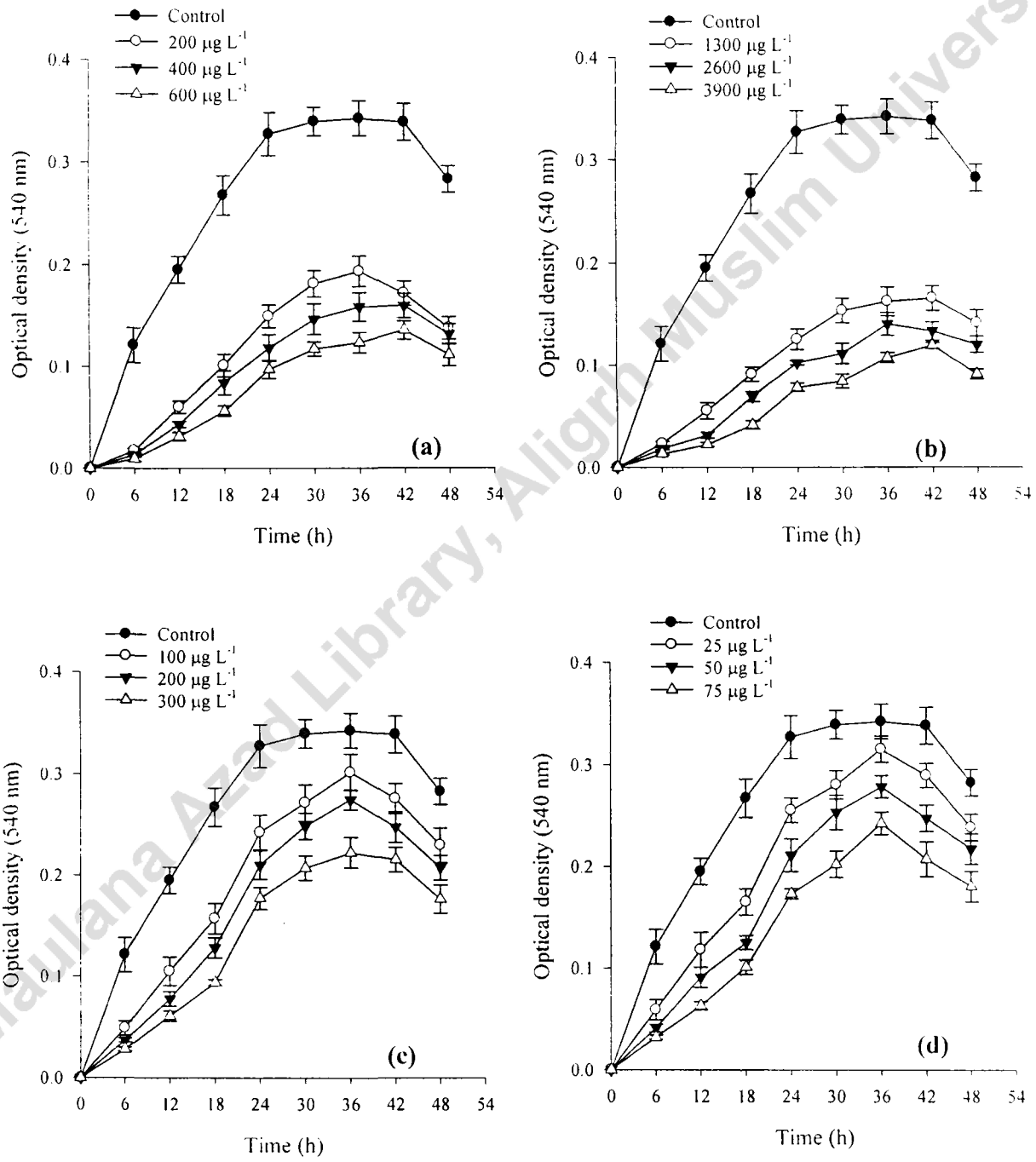


Fig. 35: Impact of recommended (○), double (▼) and three times more (Δ) of recommended rates of fipronil (a), pyriproxyfen (b), imidacloprid (c) and thiamethoxam (d) on *Bradyrhizobium* strain MRM6 (in terms of optical density) grown in minimal salt agar medium

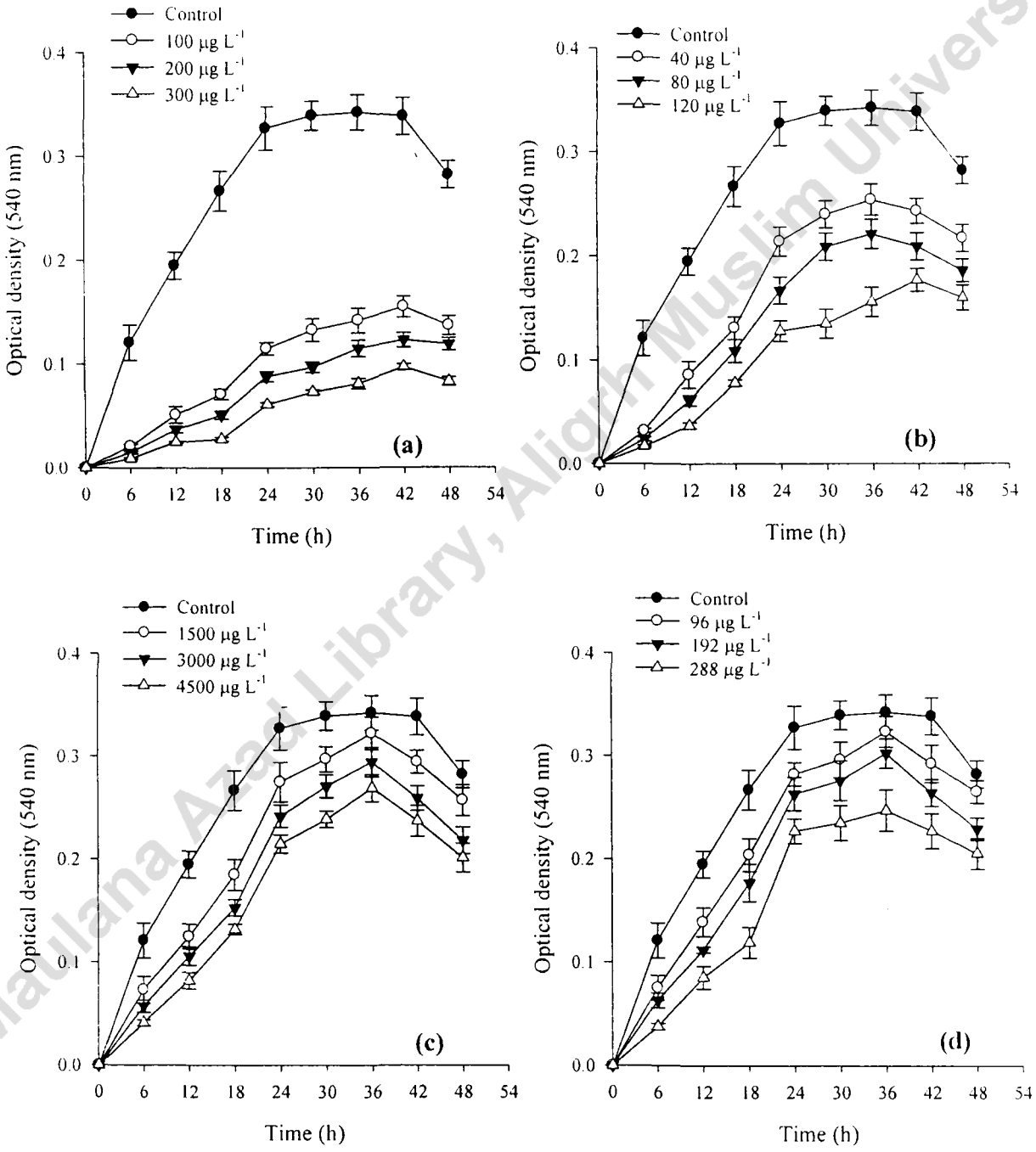


Fig. 36: Impact of recommended (○), double (▼) and three times more (Δ) of recommended rates of tebuconazole (a), hexaconazole (b), metalaxyl (c) and kitazin (d) on *Bradyrhizobium* strain MRM6 (in terms of optical density) grown in minimal salt agar medium

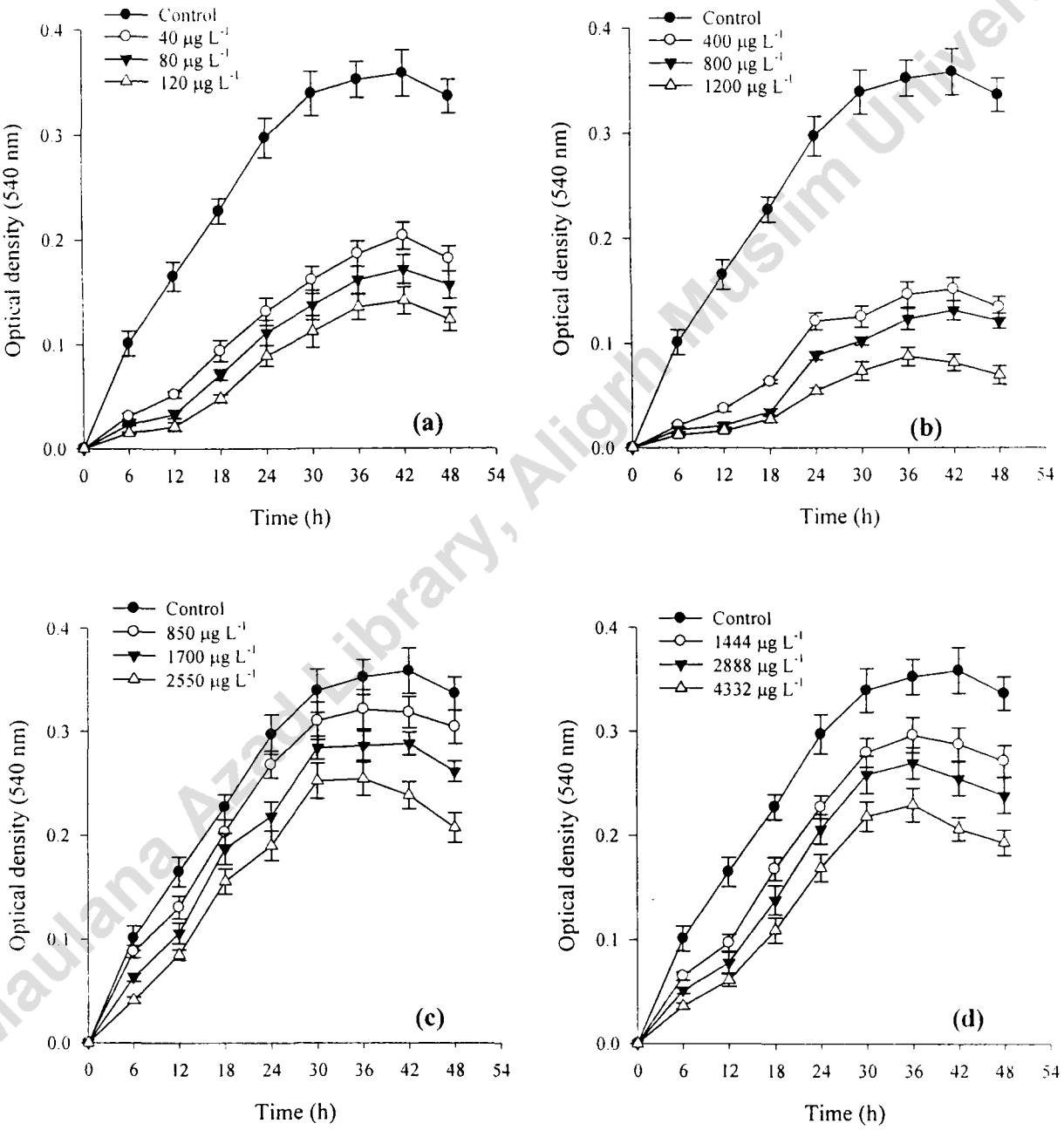


Fig. 37: Impact of recommended (○), double (▼) and three times more (Δ) of recommended rates of quizalafop-p-ethyl (a), clodinafop (b), metribuzin (c) and glyphosate (d) on *Rhizobium* strain MRL3 (in terms of optical density) grown in minimal salt agar medium

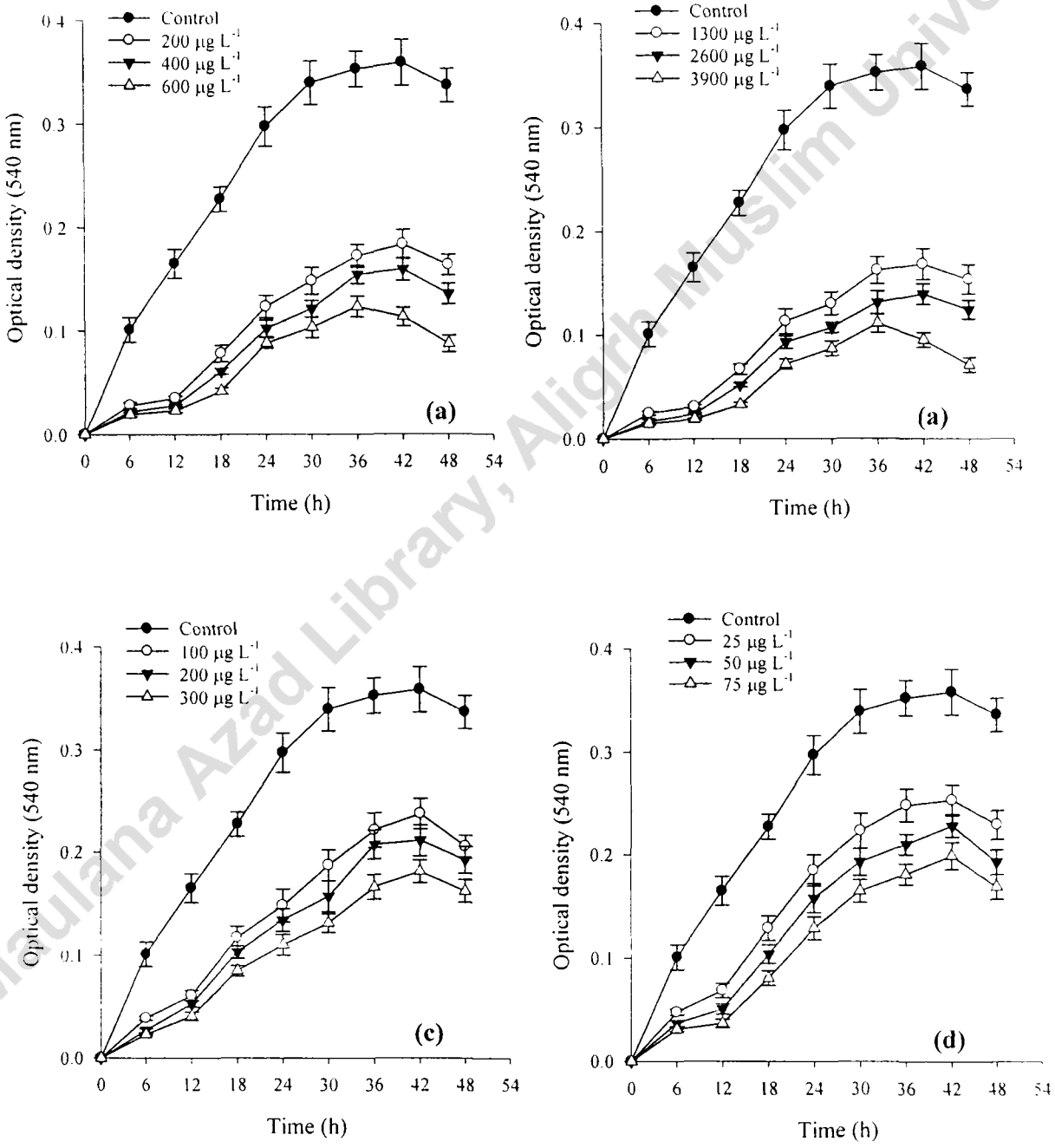


Fig. 38: Impact of recommended (○), double (▼) and three times more (Δ) of recommended rates of fipronil (a), pyriproxyfen (b), imidacloprid (c) and thiamethoxam (d) on *Rhizobium* strain MRL3 (in terms of optical density) grown in minimal salt agar medium

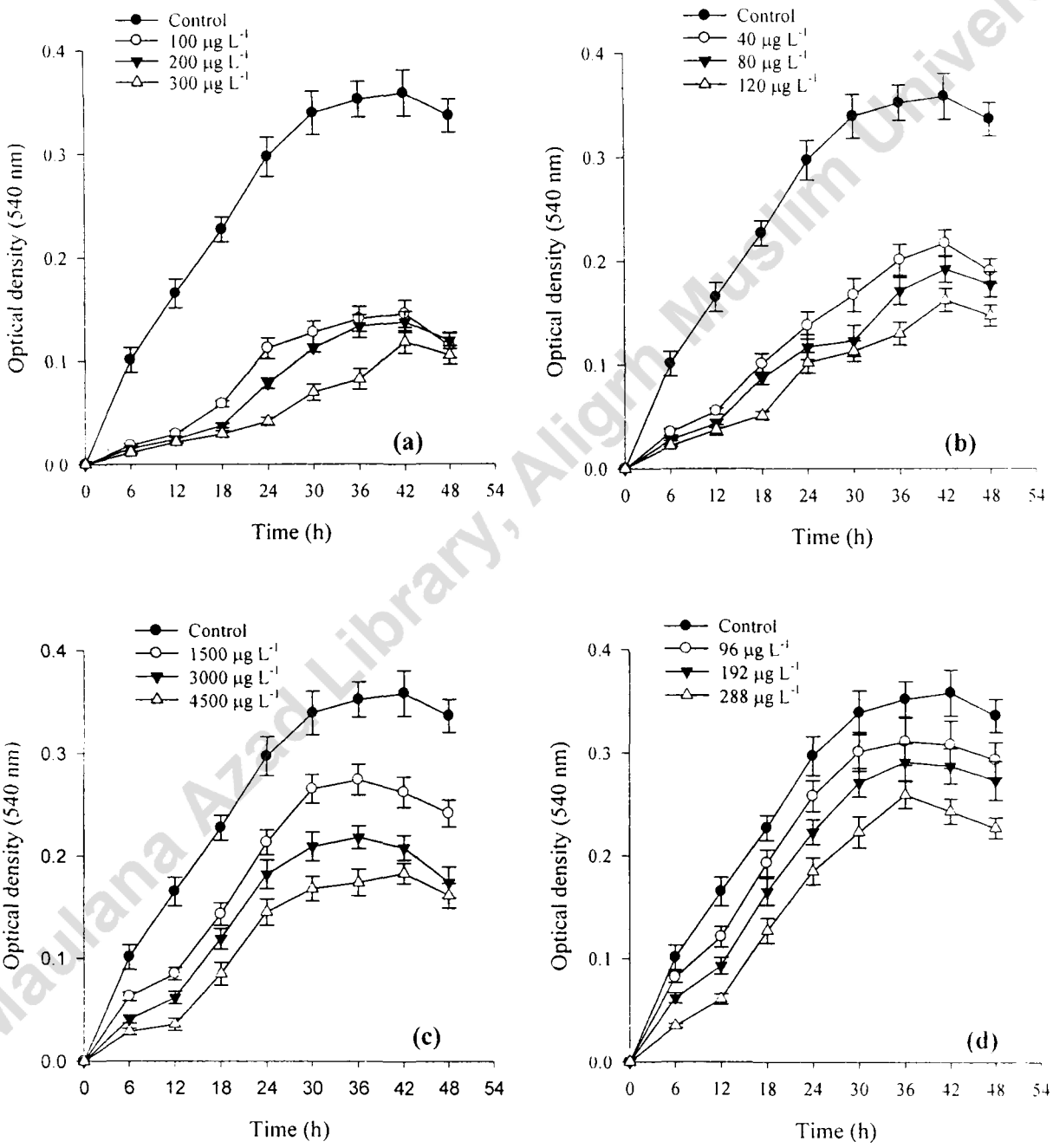


Fig. 39: Impact of recommended (○), double (▼) and three times more (Δ) of recommended rates of tebuconazole (a), hexaconazole (b), metalaxyl (c) and kitazin (d) on *Rhizobium* strain MRL3 (in terms of optical density) grown in minimal salt agar medium

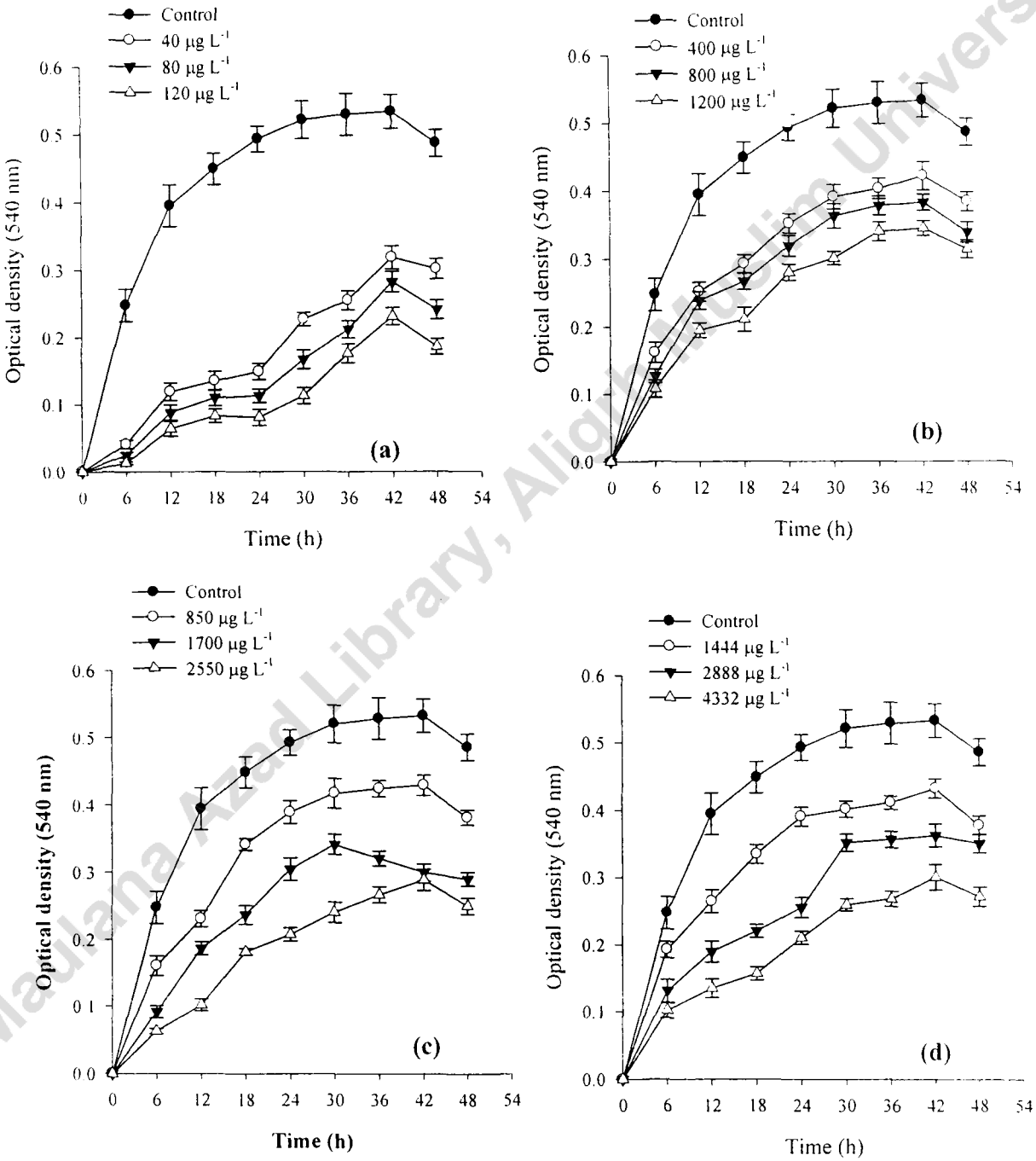


Fig. 40: Impact of recommended (○), double (▼) and three times more (Δ) of recommended rates of quizalafop-p-ethyl (a), clodinafop (b), metribuzin (c) and glyphosate (d) on *Pseudomonas aeruginosa* strain PS1 (in terms of optical density) grown in minimal salt agar medium

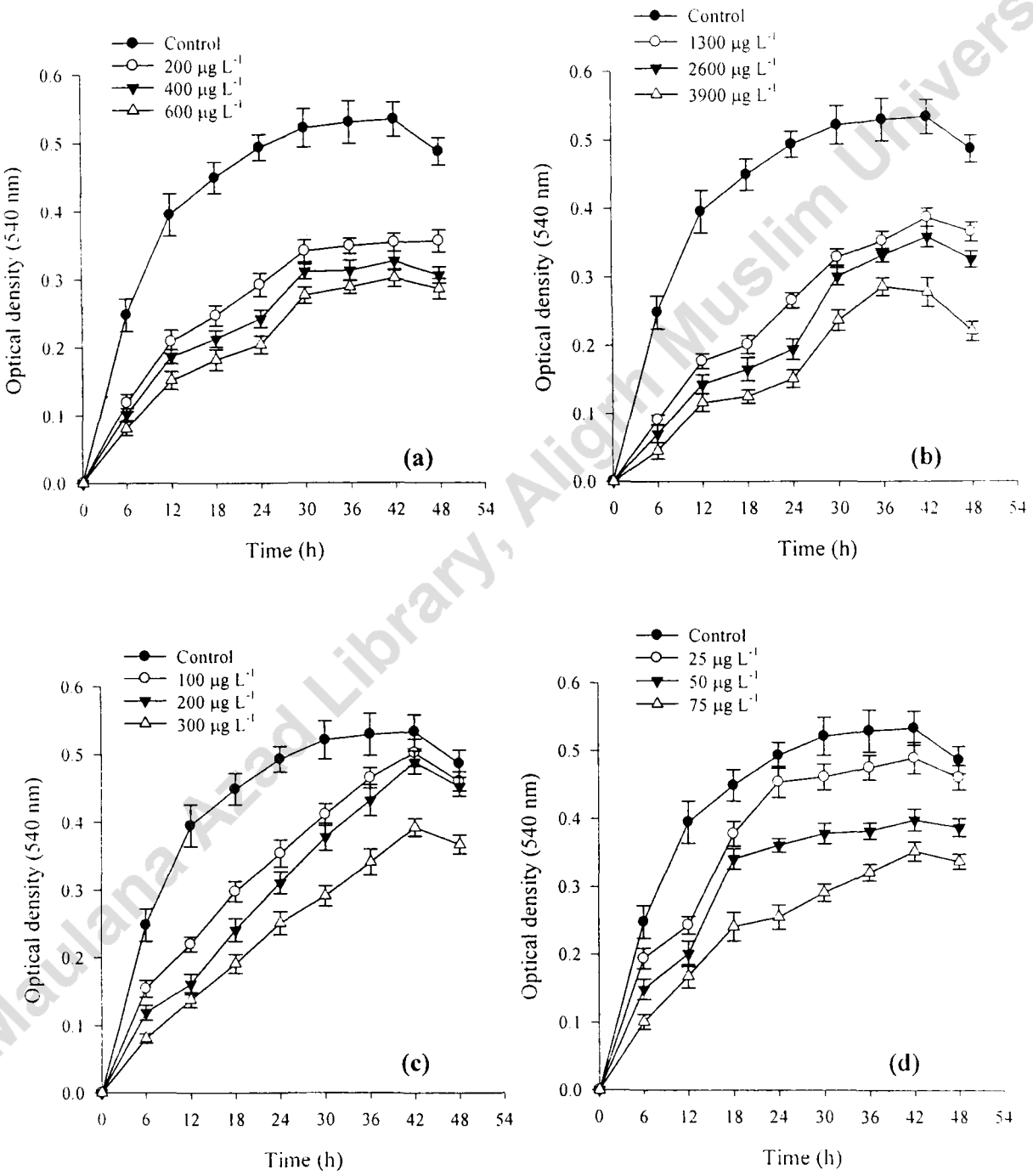


Fig. 41: Impact of recommended (○), double (▼) and three times more (Δ) of recommended rates of fipronil (a), pyriproxyfen (b), imidacloprid (c) and thiamethoxam (d) on *Pseudomonas aeruginosa* strain PS1 (in terms of optical density) grown in minimal salt agar medium

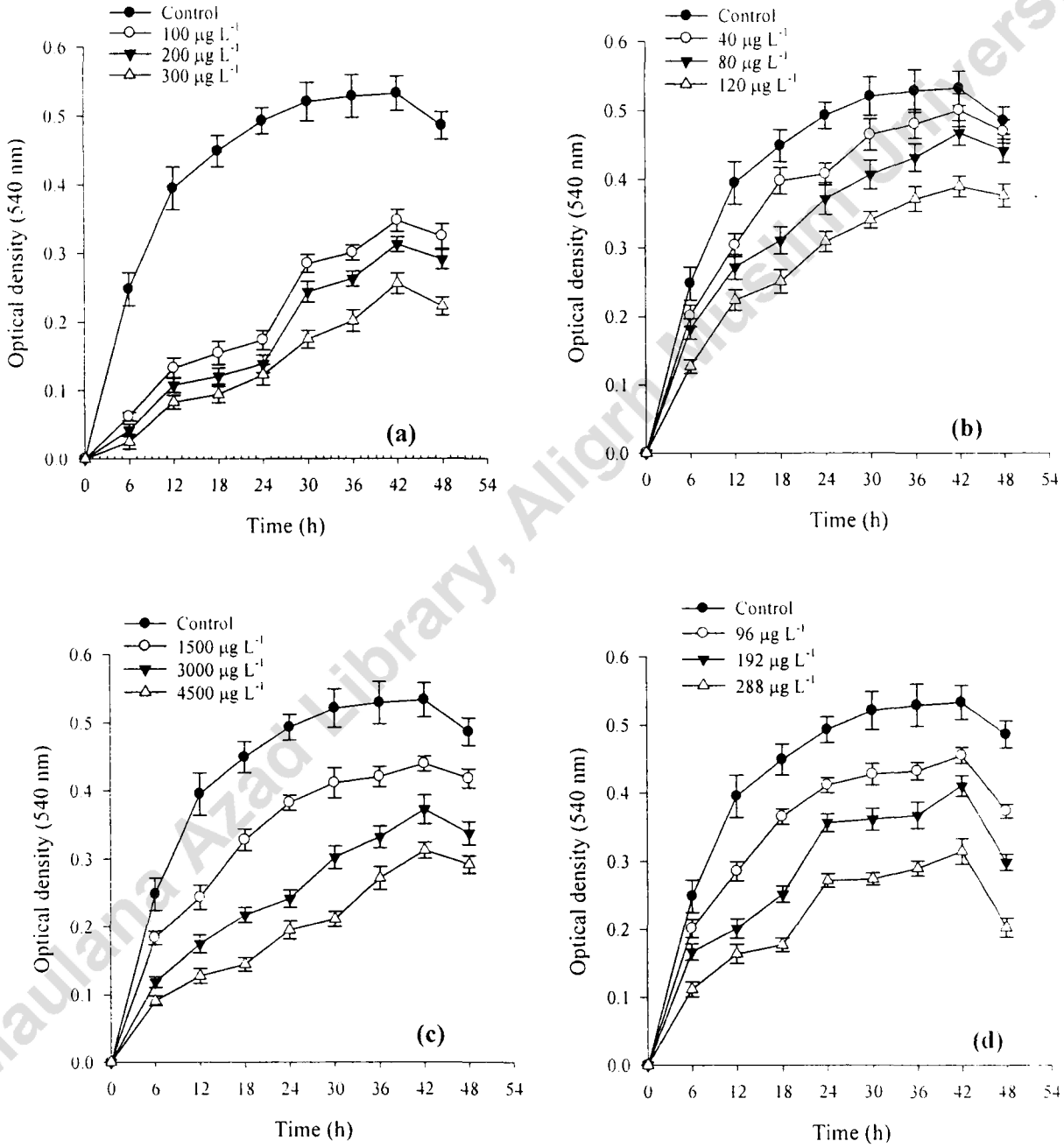


Fig. 42: Impact of recommended (○), double (▼) and three times more (Δ) of recommended rates of tebuconazole (a), hexaconazole (b), metalaxyl (c) and kitazin (d) on *Pseudomonas aeruginosa* strain PS1 (in terms of optical density) grown in minimal salt agar medium

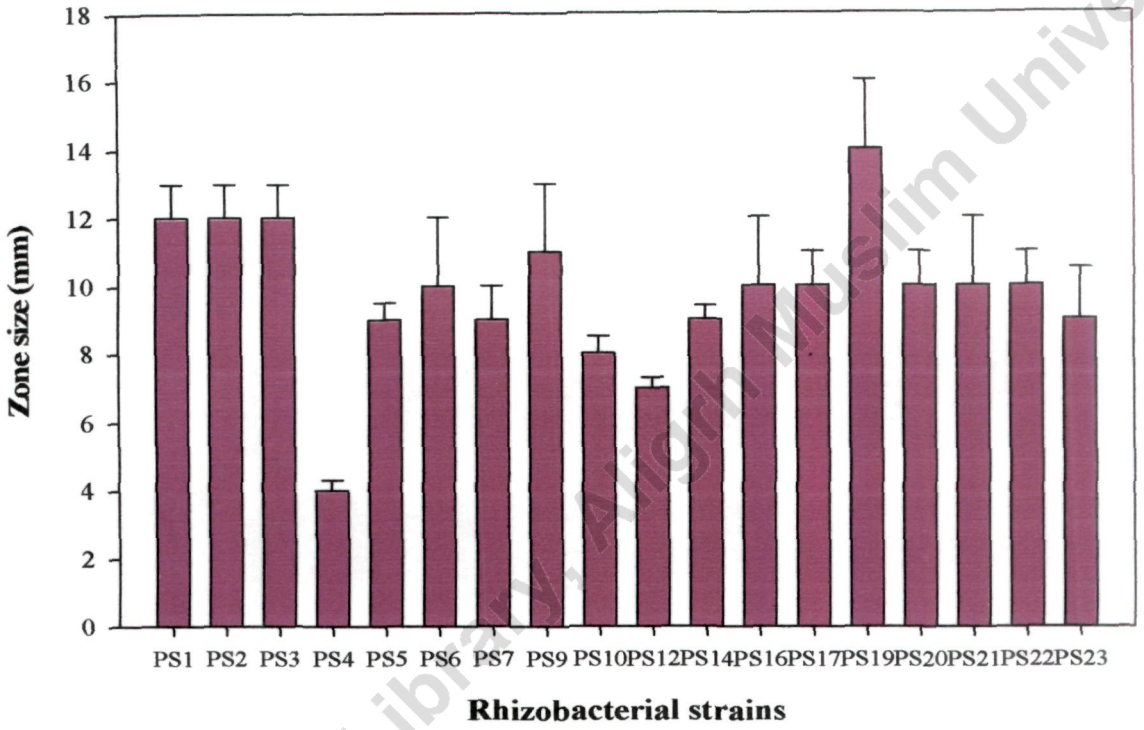


Fig. 43: Zone of P solubilization on solid Pikovskaya medium produced by phosphate solubilizing bacteria after seven days of incubation

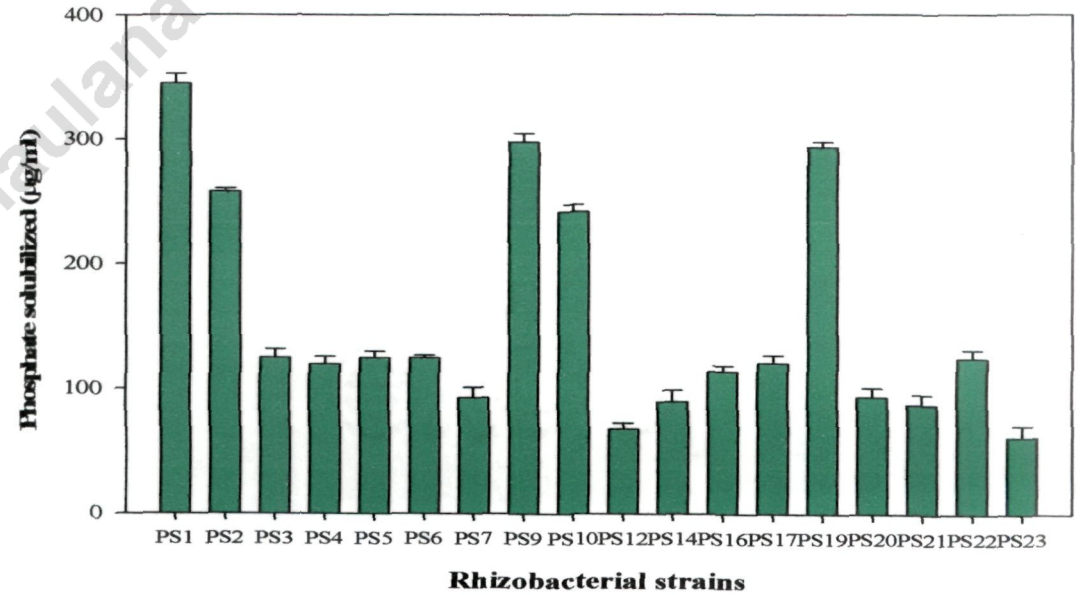


Fig. 44: *In vitro* solubilization of tri-calcium phosphate by phosphate solubilizing bacteria after seven days of incubation

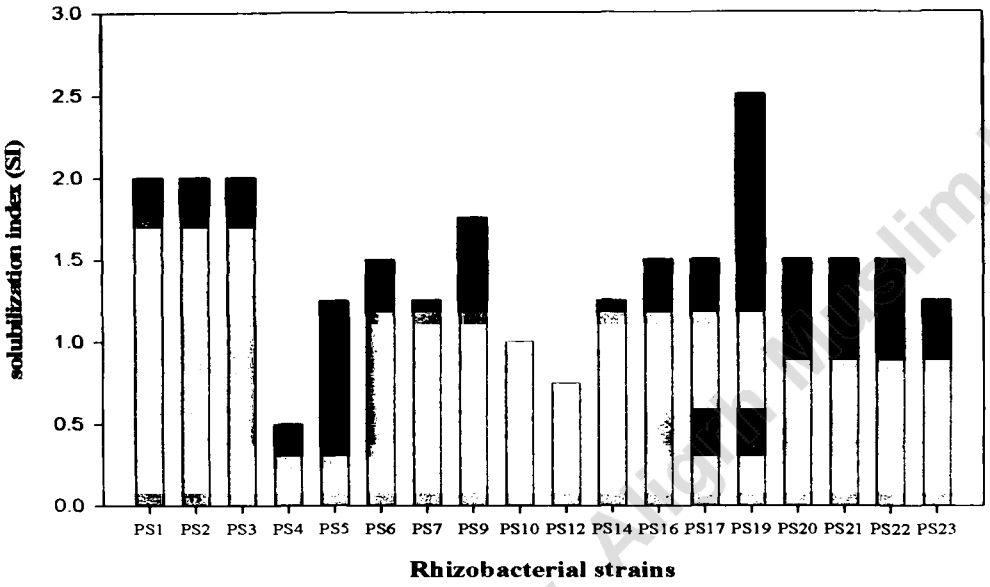


Fig. 45: Solubilization index of phosphate solubilizing bacterial strains

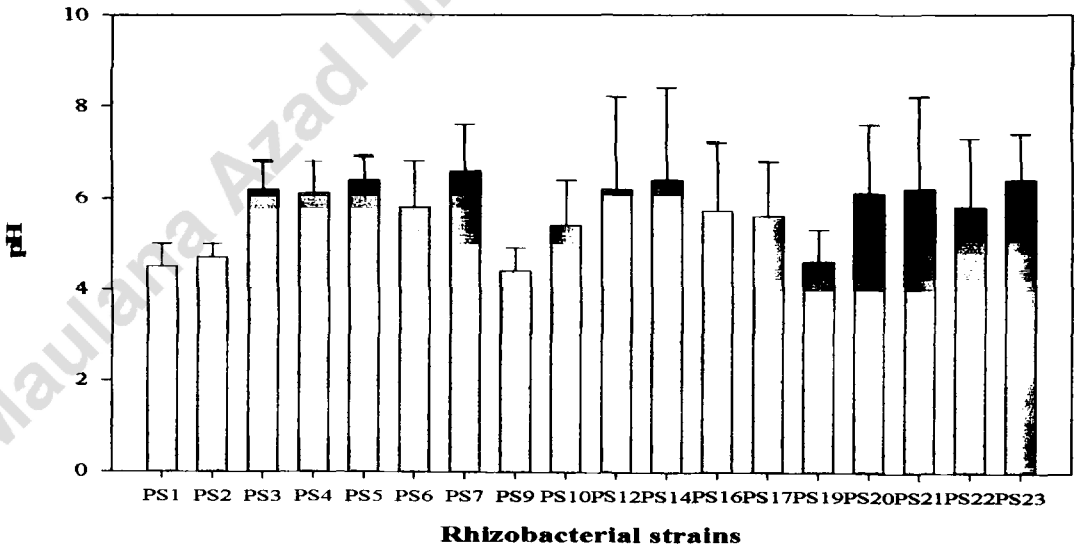


Fig. 46: Change in pH following solubilization of tri-calcium phosphate by rhizobacterial strains after seven days of incubation

Plates

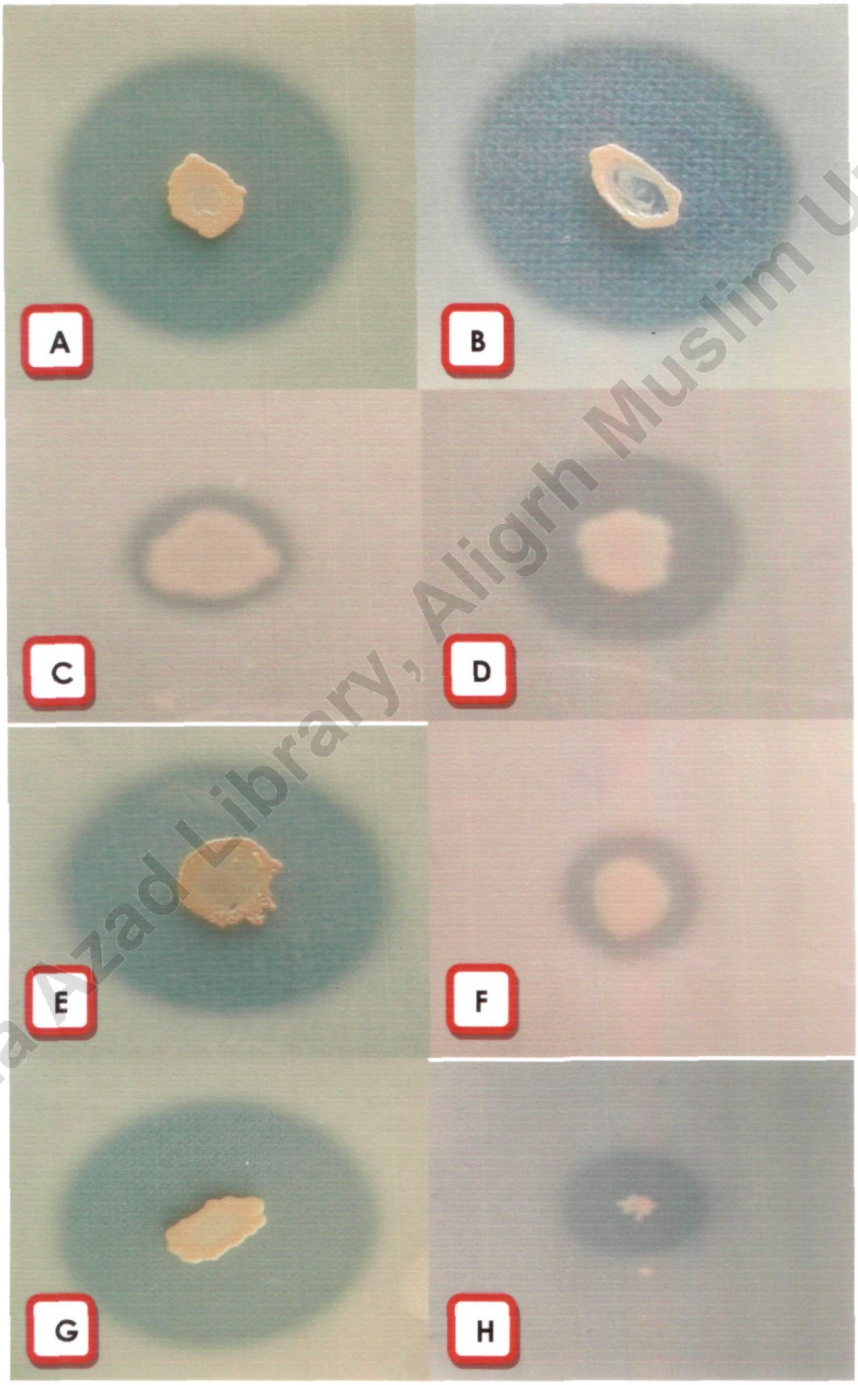


Plate 1: Plant growth promoting rhizobacteria displaying the zone of P solubilization on Pikovskaya agar (A) *Pseudomonas aeruginosa* PS1 (B) *Enterobacter asburiae* PS2 (C) *Bacillus* sp. PS4 (D) *Bacillus* sp. PS6 (E) *Pseudomonas putida* PS9 (F) *Bacillus* sp. PS14 (G) *Klebsiella* sp. PS19 (H) *Bacillus* sp. PS23

Plate 2: Plant growth promoting rhizobacteria expressing PGP traits (A) siderophore production on CAS agar plates by *Mesorhizobium* strain MRC4 and (B) *Pseudomonas aeruginosa* PS1, (C) Hydrogen cyanide (HCN) production by *Rhizobium* strain MRP1 (i) control (ii) HCN production

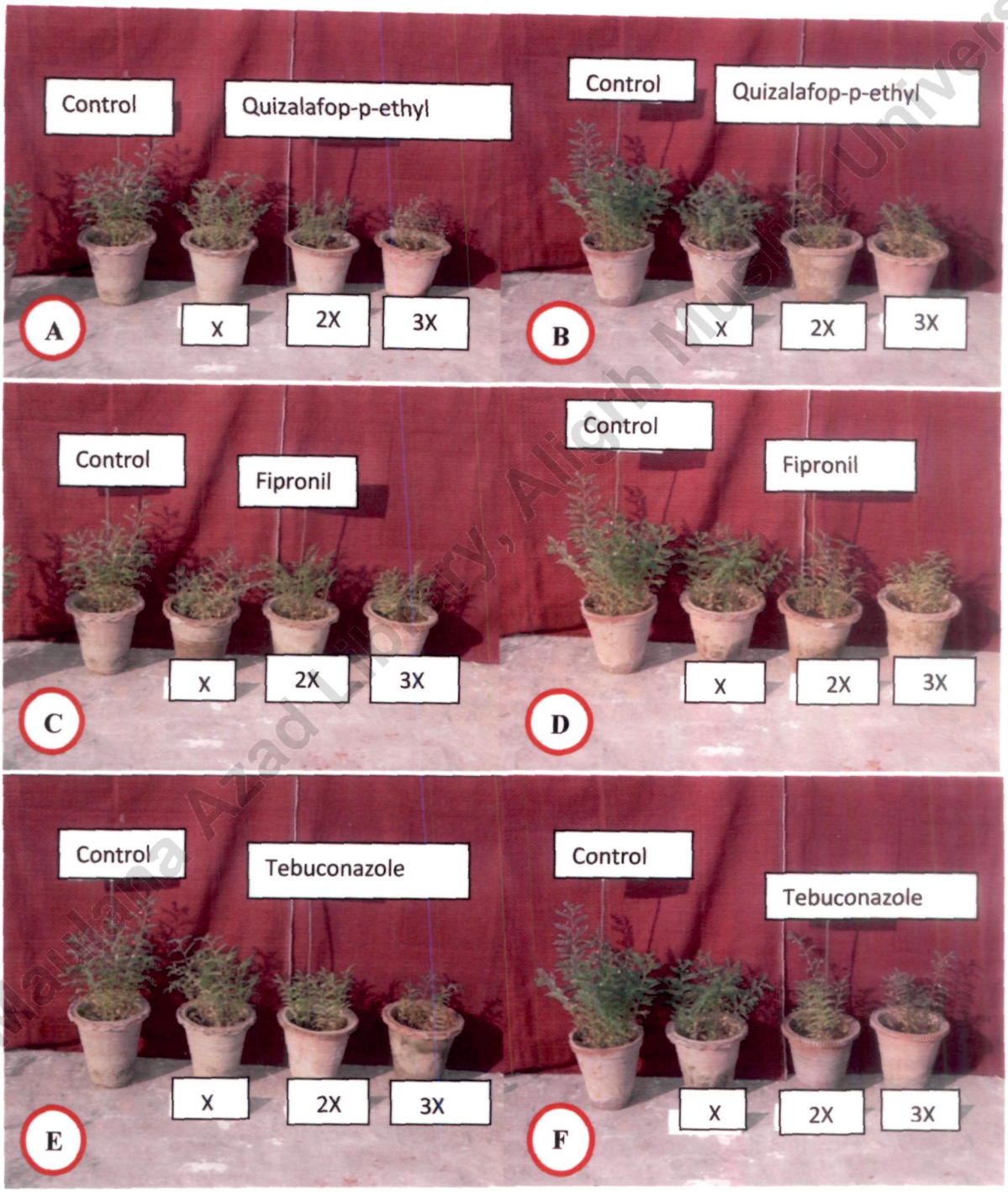


Plate 3: Effect of three concentrations of quizalafop-p-ethyl, fipronil and tebuconazole in absence (A, C and E) and presence of bioinoculant MRC4 (B, D and F) on the growth of chickpea plants

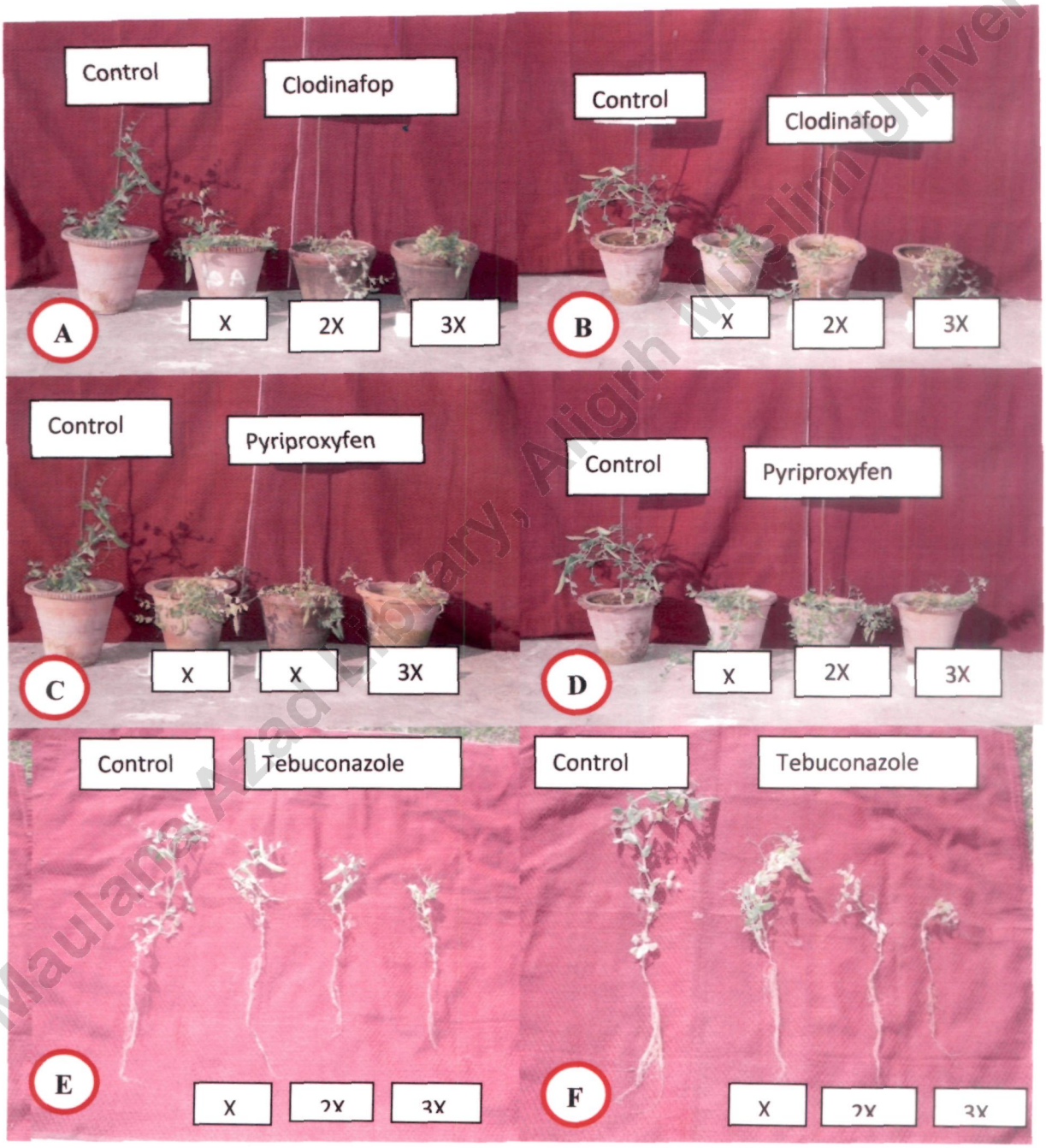


Plate 4: Effect of three concentrations of clodinafop, pyriproxyfen and tebuconazole in absence (A and C) and presence of bioinoculant MRP1 (B and D) on the growth of pea plants. Effect of tebuconazole on root and shoot length and nodulation (E-without inoculant and F- with inoculant) of pea plants

Plate 5: Effect of pesticide tolerant *Bradyrhizobium* strain MRM6/ *Pseudomonas aeruginosa* strain PS1 on the performance of greengram grown in the soil treated with different concentrations of pesticides

A and D: Growth in the absence of bio-inoculant; B and E: Growth in the presence of *Bradyrhizobium* strain MRM6; C and F: Growth in the presence of *Pseudomonas aeruginosa* strain PS1

Plate 6: Effect of clodinafop (A) and fipronil (B) on root, shoot and nodulation on greengram

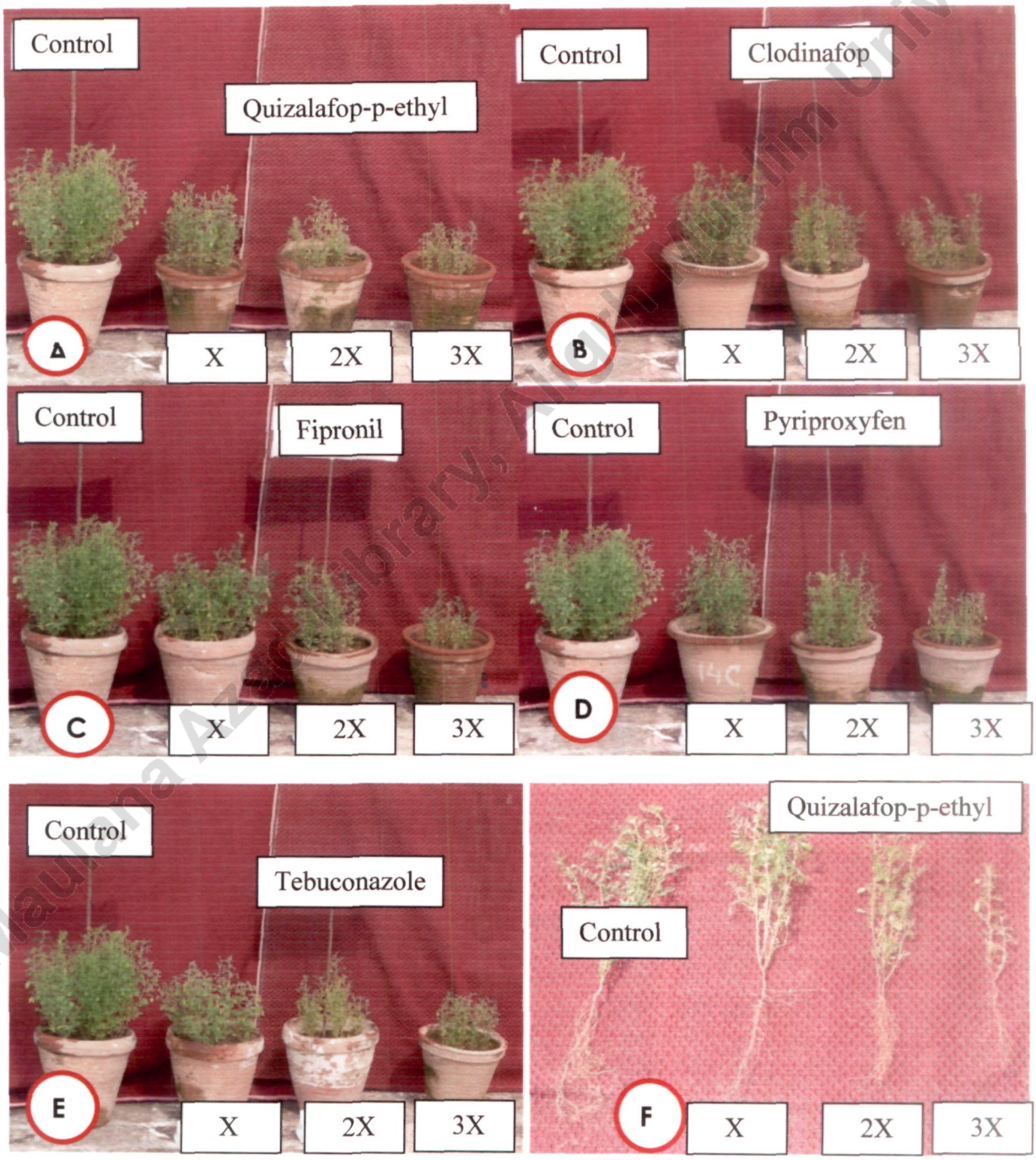


Plate 7: Effect of three concentrations of (A) quizalafop-p-ethyl, (B) clodinafop, (C) fipronil, (D) pyriproxyfen and (E) tebuconazole on the growth and nodulation of lentil plants

Plate 8: Nodulation distribution on root systems of chickpea (A) and pea (B) in the presence of bioinoculants

Plate 9: Nodulation distribution on root systems of greengram in presence of *Pseudomonas aeruginosa* strain PS1 (A) and without inoculant (B)

Discussion

5.1 Microbial diversity of rhizospheric soils of different legume and non-legume crops

Microbial communities of soils play pivotal roles in various biogeochemical cycles and influence the fertility of soils. In addition, soil microflora influence above-ground ecosystems by providing nutrients to plants; improve soil structures and consequently, affect soil health (Kirk et al., 2004, Gholami et al., 2009). Such microbes are also involved in many soil process including decomposition (Degelmann et al., 2009), nutrient mobilization and mineralization (Adesemoye et al., 2008), release of nutrients (Ponmurugan and Gopi, 2006), nitrogen fixation (Hara et al., 2009), phosphate solubilization (Katiyar and Goel, 2003, Kumar et al., 2008, Khan et al., 2009a), denitrification (Kim et al., 2008), bioremediation (Rani et al., 2009, Khan et al., 2009b) and suppression of soil borne phytopathogens (Rameshkumar and Nair, 2009). Accordingly, the microbial diversity changes in response to environmental stress (Atlas, 1984) showing both tendencies: (a) increase in diversity by selective toxicity, eliminating dominant organism and (b) diversity decreases by elimination of many species due to toxicity or increase in particular populations. As an example, Torsvik et al. (1997) observed a frequent decrease in microbial diversity in perturbed soil due to agriculture, as compared to conventional environments. Soil microbial communities in general, are subjected to a range of factors that can broadly be classified as stress factors (i.e. those that remain constantly limiting for growth of organisms and do not change markedly over time) and disturbance factors (i.e. those that involve rapid changes and often involve destruction of organism biomass). While stress and disturbance are both strong drivers of the microbial community, the effects of these factors largely operate independently from one another (Williamson and Wardle, 2007). Furthermore, microbial diversity varies greatly from soil to soil or plant genotype type to genotype. Therefore, more commonly, interest lies in understanding the structural and functional composition of soil microbial communities (Tarafdar and Claassen, 1988, Nannipieri, 1994, Dilly and Munch, 1998). More typically, however, soil microbiologists are interested in assaying the effect of a practice, process, or disturbance on the activity or composition of the soil microbial community. Thus, methods must be utilized that can detect or describe as broad a spectrum of the community of interest as possible, and also have the potential to delimit one community from another.

In the present study, viable counts of diverse microbial communities including phosphate solubilizers and fungal populations inhabiting different rhizospheric soils of pea, chickpea, lentil, greengram and mustard plants grown at experimental fields of Faculty of Agricultural Sciences, AMU, Aligarh, India were determined. A significant variation in microbial diversity in different rhizospheric soils of legume (pea, chickpea, lentil and

greengram) and non-legume (mustard) crops was observed. Generally, the microbial populations, like, total bacterial counts (4.3×10^7) and total fungal populations (1.9×10^5) were higher in the soil samples collected from mustard rhizosphere compared to legume rhizospheres; the least microbial count was however, recorded in greengram rhizosphere. The variation in heterogeneous microbial populations in tested rhizospheric soils may probably be due to the changes in physico-chemical properties such as, pH, temperature, moisture content, organic matter content (Burdman et al., 2001, Kennedy et al., 2004, Kennedy et al., 2005) of soils and the nutrients exuded by different plant species (Zak et al., 2003, Broeckling et al., 2008).

5.2 Characterization and functional diversity of PGPR

Plant growth-promoting rhizobacteria (PGPR), free-living, soil-borne bacteria which, when applied to soils, seeds or crops facilitate the growth of plants by providing nutrients (Chen et al., 2008, Wani et al., 2008) or reduce the damage caused by soil-borne plant pathogens (Kloepper et al., 1980, Saravanakumara et al., 2007). Beneficial, root colonizing, rhizosphere bacteria, the PGPR should exhibit the ability to colonize the root, survive and multiply in rhizosphere in competition with other microorganisms, express their plant growth promoting/protection (PGP) activities and ultimately should promote plant growth. For this reason, before PGPR are introduced to check their efficacy in soil/plant systems, they must be evaluated for their PGP activities *in vitro*.

Eventhough, the exact mechanisms by which the PGPR facilitate plant growth are not fully understood, yet they are believed to promote the growth of plants by numerous direct or indirect mechanisms (Glick, 1995). Direct improvement of plant growth may be exerted through several mechanisms, such as biological nitrogen fixation (Remans et al., 2008b, Figueiredo et al., 2007); synthesis of siderophores, compounds that chelate iron from soil, making it available to the plant (Katiyar and Goel, 2004b, Tripathi et al., 2005, Wani et al., 2007a, Wani et al., 2008); solubilization of minerals such as phosphorous (Gupta et al., 2002, Katiyar and Goel, 2003, Khan et al., 2009a), or synthesis of plant hormones, such as auxins (Rodrigues et al., 2008, Indiragandhi et al., 2008), gibberellins (Gutie´rrez Manero et al., 2001); or plant hormone regulators, such as ACC deaminase (Ganesan, 2008, Jiang et al., 2008), an enzyme that decreases endogenous concentrations of ethylene. The indirect promotion of plant growth on the other hand occurs when rhizobacteria lessen or prevent the deleterious effects of phytopathogenic organisms (Zehnder et al., 2000). Therefore, the PGPR belonging to phosphate solubilizing and nitrogen fixing groups were isolated in this study and

evaluated for their diversity in terms of plant growth promoting activities in order to explore such PGPR for the growth promotion of legumes like, chickpea, pea, lentil and greengram.

In this study, a total of 250 PGPR strains were isolated both from the nodules produced on root systems of legumes like, chickpea, pea, lentil and greengram and rhizospheric soils of mustard grown at the experimental fields of Faculty of Agricultural Sciences, AMU, Aligarh, India. The isolated PGPR strains were identified using standard morphological and biochemical tests (Holt et al., 1994). Later, most promising phosphate solubilizing bacterial strains (PS1, PS2, PS9 and PS19) recovered from mustard rhizosphere though produced similar pigments on Pikovskaya plates but when subjected to 16S rDNA sequencing (Macrogen Inc., Seoul, Korea) were identified differently as *Pseudomonas aeruginosa* (Gene Bank accession number FJ705886), *Enterobacter asburiae* (FJ705887), *Pseudomonas putida* (FJ705888) and *Klebsiella sp.* (FJ705889), respectively. The rDNA sequence of *Pseudomonas aeruginosa* PS1, *Enterobacter asburiae* strain PS2, *Pseudomonas putida* PS9 and *Klebsiella sp.* PS 19 were found 99, 99, 99 and 98% similar to those of *Pseudomonas aeruginosa* strain MW3AC (accession number GQ180118), *Enterobacter asburiae* strain J2S4 (accession number EU221358), *Pseudomonas putida* strain ATCC 17514 (accession number AF094741) and *Klebsiella* sp. WW2 (accession number EF433545) respectively, stored in NCBI database.

Further, based on the growth promoting potentials of each PGPR strain assayed under *in vitro* conditions, *Mesorhizobium* strains from chickpea nodules were categorized into four PGP groups where group I included four strains (MRC1, MRC4, MRC7 and MRC10) with five PGP traits, followed by group II, which had three strains (MRC3, MRC9 and MRC12); all of which were positive for ammonia, HCN and IAA, group III also included three strains (MRC5, MRC6 and MRC11) which were positive to ammonia and IAA and group IV contained only one strain (MRC14) which synthesized IAA only. Similarly, the *Rhizobium* spp. isolated from pea and lentil and *Bradyrhizobium* strains recovered from greengram were grouped into three PGP types, while phosphate solubilizing rhizobacterial strains were divided into four PGP groups. Generally, the production of ammonia and IAA were the most prevalent PGP traits in rhizobial strains. In contrast, the most frequent traits of phosphate solubilizers were the production of ammonia, IAA and exopolysaccharides. The production of such growth regulating substances by plant growth promoting rhizobacteria like *Mesorhizobium* (Wani et al., 2008), *Rhizobium* (Wani et al., 2007b), *Bradyrhizobium* (Wani et al., 2007a), *Pseudomonas* (Joseph et al., 2007, Jeon et al., 2003, Botelho et al., 1998) and other rhizobacteria (Saravanan et al., 2007, Jha and Kumar, 2007, Rodrigues et al., 2008) have also been reported.

The increased use of agrochemicals including pesticides has led to the frequent and deliberate contamination of agricultural soil. The potential danger of these chemicals to the rhizospheric organisms and associated biotic processes are governed by the rate of application, the toxicity and activity spectrum of pesticides and the persistence and availability of chemicals in soils. Many pesticides are known to produce deleterious effects on the populations and activity of beneficial soil microorganisms that catalyze various biological processes important to soil fertility and plant growth(Moorman, 1989, Srinivas et al., 2008). Furthermore, the introduction of new classes of pesticides further warranted the new research on the potential effects of these chemicals (Wardle and Parkinson, 1990, Cernakova et al., 1991). Studies on the effect of various agrochemicals have largely concentrated on either plants or on soil microflora. However, literature is scanty where both plants and beneficial microflora have been subjected to the pesticide application together. Through this perspective, this study was designed to evaluate the toxic effects of 12 pesticides including four each of herbicides (quizalafop-p-ethyl, clodinafop, metribuzin, glyphosate), insecticides (fipronil, pyriproxyfen, imidacloprid, thiamethoxam) and fungicides (tebuconazole, hexaconazole, metalaxyl and kitazin) groups at recommended, double and three times more of recommended rates on the survival and *in vitro* plant growth promoting activities of such rhizobacteria and also on the performance of legume crops grown in sandy clay loam soils treated with selected pesticides.

In the present study, tolerance level of the PGPR strains to the varying concentrations of herbicides, insecticides and fungicides were assessed under *in vitro* conditions. The present experiment was set up in order to identify the PGPR strains capable of tolerating high level of herbicides, insecticides and fungicides which could later be exploited as bioinoculant for their application in soils contaminated heavily with pesticides. While analyzing the tolerance of different isolates against the consistently increasing concentration from recommended dose to higher one upto three times of each pesticide, some rhizobacterial isolates displayed an exceptionally higher degree of tolerance to each of the three groups of pesticides tested. Among the N_2 fixers, the degree of tolerance in terms of MRL (maximum resistence level) of rhizobial isolates recovered from pea, chickpea, lentil and greengram nodules, ranged from 800 to 3200 µg/ml. Additionally, phosphate solubilizers also exhibited abnormally higher tolerance to 12 pesticides whose MRL values ranged from 600 to 3200 µg/ml. Of these, *Pseudomonas aeruginosa* (strain PS1), *Enterobacter asburiae* (strain PS2), *Pseudomonas putida* (strain PS9) and *Klebsiella* sp. (strain PS19) were comparatively most tolerant to the tested pesticides than the other bacterial strains. In similar studies, Gram negative bacteria

have also shown resistance to other pesticides. For instance, the maximum tolerant concentrations of different organophosphorus pesticides for both resistant strains of *Pseudomonas* and *Flavobacterium* species isolated from polluted sites were 250, 4000 and 8000 μg ml^{-1} of guthion, methyl parathion and dimethoate, respectively (Nazarian and Mousawi, 2005). Likewise, *Rhizobium* sp. specific to chickpea and *Rhizobium* sp. specific to greengram tolerated aldrin upto 2000 μg ml^{-1} (Juneja and Dogra, 1978). Moreover, Boldt and Jacobsen (1998) also reported a variation in the MRLs of *Pseudomonas* strains to sulfonylurea herbicides (e.g. metsulfuron methyl, chlorsulfuron and thifensulfuron methyl). Among the herbicides, metsulfuron methyl was more toxic compared to other herbicides and order of toxicity was: metsulfuron methyl > chlorsulfuron > thifensulfuron methyl. The variation in tolerance to pesticide by PGPR could probably be due to the fact that rhizobacteria adopt different strategies to overcome the toxic effects of pesticides and such mechanisms include: biodegradation (Yang and Lee, 2008) and enzymatic hydrolysis (Dumas et al., 1989, Herman et al., 2005) of pesticide by PGPR strains. For instance, organophosphorus hydrolase (OPH), an enzyme isolated from *Pseudomonas diminuta* MG and *Flavobacterium* sp. strain ATCC 27551, possess the ability to hydrolyze different organophosphorus insecticides (Dumas et al., 1989). Hydrolysis of organophosphorus compounds by OPH dramatically reduced their toxicity (DiSoudi et al., 1999). Similarly, dicamba monooxygenase (DMO), an enzyme extracted from *Pseudomonas maltophilia* strain DI-6, completely inactivated the herbicidal activity of dicamba (Herman et al., 2005). Our study however, showed that the resistance level of the selected rhizobacterial strains was considerably quite high for the pesticides.

Usually, plant growth promotion by PGPR is influenced by the production of phytohormones as well as essential nutrients. In addition, they can also utilize the agrochemicals as C and N sources (Edwards et al., 1992, Marihal et al., 2009) and consequently reduce the toxicity of pesticides in pesticides stressed soils. As a result of this activity, the PGPR strains are likely to promote the growth of crops including legumes cultivated in pesticides polluted sites. Among the phytohormones, indole acetic acid (IAA) and its analogues, synthesized from tryptophan, primarily in leaf primordial, young leaves and developing seeds, are the main auxin in most plants, controlling many important physiological processes including cell enlargement and division, tissue differentiation, root initiation, root growth inhibition, increased growth rate, phototropism, geotropism and apical dominance (Frankenberger and Arshad, 1995, Khan et al., 2009a). Bacterial IAA has the potential to interfere with any of these processes by input of IAA into the plant's auxin pool.

Effect of IAA (both bacterial and plant origin) on plants however, depends upon the amount of IAA produced and the sensitivity of the plant tissue to changes in IAA concentration. A root, for instance, is one of the plant's organs that is most sensitive to fluctuations in IAA. In this study, of the total 250 PGPR strains, a total of 21% bacterial strains resistant to 12 pesticides were screened for IAA production. The strains of *Mesorhizobium* (N= 11), *Bradyrhizobium* (N= 9), *Rhizobium* (N= 7) isolated from pea and *Rhizobium* (N= 8) from lentil nodules and a total of 18 phosphate solubilizers (*Pseudomonas*, *Bacillus*, *Enterobacter* and *Klebsiella*) produced a substantial amount of IAA in LB broth supplemented with 100 µg/ml tryptophan.

The potential of N_2 fixing and phosphate solubilizing bacteria to tolerate considerably higher amounts of herbicides, insecticides and fungicides and to express PGP traits in pesticides stressed soils, make them one of the most suitable choices to select them as bioinoculants which could be used to enhance the crop yields when applied to derelict soils. Therefore, the growth regulating activities of pesticide tolerant strains in the presence of three concentrations (X, 2X and 3X) of selective groups of pesticides were assessed further. The nodule bacteria (*Mesorhizobium*, *Rhizobium* and *Bradyrhizobium*) and P-solubilizers (*Pseudomonas*, *Bacillus*, *Enterobacter* and *Klebsiella*) produced a varying amount of IAA in LB broth supplemented with a fixed (100 µg/ml) concentration of tryptophan even in the presence of pesticides. Differences in the synthesis of IAA among rhizobacterial strains however, can be attributed to the involvement of various biosynthetic pathways, location of the genes involved, regulatory sequences, the presence of enzymes to convert active free IAA into conjugated forms and changing environmental conditions (Patten and Glick 1996). Interestingly, the production of rhizobacterial IAA decreased progressively with increase in pesticide concentrations from X to 3X. The production of IAA by the plant growth promoting rhizobacterial strains in this study however indicated that the IAA synthesis was adversely affected under pesticide stress. In a similar study, Wani et al. (2005) also reported a substantial decline in IAA secretion by *Serratia*, *Pseudomonas* and *Bacillus* when cultured in the presence of phorate at the rate of 100 and 500 µg /ml under *in vitro* conditions. Similar evidence of phytohormone production by *Mesorhizobium* (Wani et al., 2008), *Bradyrhizobium* (Wani et al., 2007a) and *Rhizobium* (Wani et al., 2007b), *Bacillus* (Singh et al., 2008, Zaidi et al., 2006), *Pseudomonas* (Poonguzhali et al., 2008, Shaharoona et al., 2008, Indiragandhi et al., 2008, Ganesan, 2008), *Enterobacter* (Kumar et al., 2008) and other

rhizosphere bacteria (Selvakumar et al., 2008, Jiang et al., 2008) in conventional and stress medium is reported.

Siderophore is yet another important metabolite released by the PGPR strains that indirectly affects the growth of plants. In the present study, among the selected 53 rhizobacterial strains, four strains belonging to genera *Mesorhizobium*, three strains to rhizobia of pea and *Bradyrhizobium,* four strains belonging to rhizobia of lentil origin and 10 strains of phosphate solubilizers produced siderophore on CAS agar plates as well as in liquid culture medium. Further, strains showing the highest production of siderophores were selected from each group to examine the effect of three concentrations of each pesticide on siderophore synthesizing ability of each rhizobacterial strain. In the presence of three concentration of each pesticide used separately, pesticide tolerant strains showed a positive siderophore activity as indicated by the development of orange colored zone on CAS agar plates after four days of growth and yielded a substantial amount of SA and DHBA in culture supernatant after extraction with ethyl acetate. The amount of both SA and DHBA however, declined progressively on increasing the concentration of herbicides (quizalafop-p-ethyl, clodinafop, metribuzin, and glyphosate), insecticides (fipronil, pyriproxyfen, imidacloprid and thiamethoxam) and fungicides (tebuconazole, hexaconazole, metalaxyl and kitazin). For instance, quizalafop-p-ethyl at 3X displayed the maximum decrease in production of SA by 35, 68, 46 and 47% and of DHBA by 48, 78, 89 and 90% for *Pseudomonas aeruginosa* PS1, *Enterobacter asburiae* PS2, *Pseudomonas putida* PS9 and *Klebsiella* sp. PS19, respectively, over their respective control.

Siderophores synthesized by microbial communities of soil supply iron to plants that possess the mechanisms for its uptake under iron-deficient conditions (Indiragandhi et al., 2008). Furthermore, siderophores chelate iron and other metals and consequently cause disease suppression by conferring a competitive advantage to biocontrol agents for the limited supply of essential trace minerals in natural habitats. Siderophores may also directly stimulate the biosynthesis of other antimicrobial compounds by increasing the availability of these minerals to the bacteria and may function in local and systemic host resistance in plants (Joseph et al., 2007; Wani et al., 2008; Sinha and Mukherjee, 2008). The ability of rhizobial strains and phosphate solubilizers to produce siderophore as observed in this study suggests that such strain could also be used as a biological control agent. Similar evidence of siderophore production by *Pseudomonas* (Dey et al., 2004, Poonguzhali et al., 2008, Rajkumar and Freitas, 2008) and *Rhizobium* (Wani et al., 2007b) is also reported.

Further, the synthesis of HCN and ammonia by the PGPR strains was tested under both absence and presence of pesticides. Among the total 53 PGPR strains, 19% of *Mesorhizobium*, 17% of *Bradyrhizobium*, 11% of pea rhizobia, 15% of lentil rhizobia and 34% of phosphate solubilizers were found positive for ammonia. Similarly, 13% of *Mesorhizobium*, 6% of *Bradyrhizobium*, 13% of pea rhizobia, 6% of lentil rhizobia and 17% of phosphate solubilizers were found positive for HCN. Cyanide and ammonia are reported to be produced by several plant growth promoting rhizobacteria from glycine and cyanogenic glycosides present in root exudates. The ammonia released by the bacterial strain plays a signaling role in the interaction between plant growth promoting bacteria and plants (Becker et al., 2002). Moreover, the ammonia released by the bacterial strain is known to increase the glutamine synthetase activity (Chitra et al., 2002). In addition, ammonia transporters found in several PGPR are thought to be involved in the re-absorption of NH_4^+ released as a result of NH_3 diffusion through the bacterial membrane (Van Dommelen et al., 1997). In a similar study, Devi et al. (2007) reported the excretion of HCN by the rhizobacterial strains into the rhizosphere. The release of HCN by rhizospheric bacteria into the soil can be toxic to subterranean animals and phytopathogenic organisms (Guo et al., 2007). Similarly, ammonia production by rhizobial strain is reported elsewhere (Wani et al., 2007a, Wani et al., 2008).

Furthermore, four strains of *Mesorhizobium*, three each of *Bradyrhizobium*, pea and lentil specific rhizobia and all of phosphate solubilizers were tested for their ability to secrete EPS. Interestingly, all the chosen bacterial strains produced exopolysaccharides when nodule bacteria and phosphate solubilizers were grown in basal medium supplemented with 5% sucrose. The most tolerant PGPR among nitrogen fixers, *Mesorhizobium* strain MRC4, *Bradyrhizobium* strain MRM6, pea specific *Rhizobium* strain MRP1, lentil specific *Rhizobium* strain MRL3 and *Pseudomonas aeruginosa* strain PS1, *Enterobacter asburiae* strain PS2, *Pseudomonas putida* strain PS9 and *Klebsiella* sp. strain PS19 among phosphate solubilizer, were also analyzed for EPS production in medium supplemented with varying concentration of herbicides, insecticides and fungicides. The most promising strains produced EPS even in the presence of every pesticide used in this study at three times of recommended dose. The EPS production is an important trait of bacteria because it provides protection to cells against desiccation, phagocytosis and phage attack and also helps in N_2 fixation by preventing high oxygen tension (Tank and Saraf, 2003). Furthermore, the bacteria producing higher amounts of EPS exhibit a stronger ability of phosphate solubilization compared to non-EPS producing strains (Yi et al., 2007). Interestingly, the amount of EPS secreted by rhizobacteria in this study increased progressively with gradual increase in pesticidal

concentrations. Though, the role of EPS is well known but why EPS increased with increasing concentration of pesticides in this study is not clear. However, the increase in EPS following increased concentration of pesticides suggests that the pesticides might have acted as inducer of EPS synthesis. The EPS so excessively synthesized by bacterial strains is likely to provide them protective advantage while inhabiting the stressed environments.

Phosphorus (P) is though a major plant nutrient limiting the plant growth but is required for various metabolic processes like energy transfer, signal transduction, macro-molecular biosynthesis, photosynthesis and respiration by plants (Fernández et al., 2007, Khan et al., 2009*a*.). Phosphorus in the soils is present both in organic and inorganic forms. Of these, organic forms, as found in humus and other organic materials including decayed plant, animal and microbial tissues, is an important reservoir of immobilized P accounting for about 20-28% of total soil P. In general, the majority of P applied exogenously or present in complex forms in soils is unavailable for uptake by plants due to its rapid rate of fixation/complex formation with other elements of soils (Goldstein, 1986, Khan et al., 2009a). Therefore, P fertilizers are applied to soil to replenish the P demands of growing plants. Since phosphatic fertilizers are expensive and the efficiency of the externally added P fertilizer is as low as about 10% (Werft and Dekkers, 1996), this has led to identify the environment-friendly and economically feasible alternative strategies for improving crop production in low or P deficient soils. In this context, plant growth promoting rhizobacteria possessing P solubilizing activity often termed phosphate solubilizing bacteria are considered as promising biofertilizers since they can supply plants with P from sources otherwise poorly available.

The PGPR strains were therefore further screened and evaluated for their P solubilizing potential using both solid and liquid Pikovskaya medium supplemented with or without pesticides. In the present study, a total of 34% rhizobacterial strains showed the phosphate solubilizing activity on solid Pikovskaya plates as detected by the formation of a clear halo around their growth. The PGPR strains also solubilized an appreciable amount of TCP in liquid Pikovskaya medium with concomitant drop in pH of the culture medium. However, the size of halo as well as TCP solubilization in Pikovskaya broth decreased progressively relative to the control (no pesticide) when cultures were grown in the presence of increasing dose of pesticides. The solubilization of insoluble P by the rhizosphere microorganisms and concurrently decrease in pH of the medium, has often been due to the secretion of organic acids (Chen et al., 2006, Ponmurugan and Gopi 2006, Park et al., 2009). In addition, the bacteria producing higher amounts of EPS exhibit a stronger ability of P-

solubilization compared to non-EPS producing strains as reported by Yi et al. (2007). This inference is further consolidated by the observation that all phosphate solubilizers produced EPS and that *Pseudomonas aeruginosa* strain PS1, *Enterobacter asburiae* strain PS2, *Pseudomonas putida* strain PS9 and *Klebsiella* sp. strain PS19 solubilizing maximum TCP in liquid medium also produced the highest concentration of EPS. In addition, the amount of P solubilized in liquid Pikovskaya medium and EPS secreted in basal medium by all selected P solubilizers was found significantly correlated (r = 0.712). Similar evidence of phosphate solubilization under conventional environment by the *Bacillus*, *Pseudomonas* and *Enterobacter* (Zaidi et al., 2006, Singh et al., 2008, Poonguzhali et al., 2008, Rajkumar and Freitas, 2008, Kumar et al., 2008) is reported. The present study suggested that the intrinsic ability of the pesticide tolerant rhizobacterial strains of expressing the production of plant growth promoting substances both in the presence and absence of pesticides could be exploited to augment the growth of plants under pesticides stressed environment.

5.3 Pesticidal toxicity to legumes and rhizoremediation studies

In modern agronomic practices, pesticides are often used to control pests of agricultural importance and consequently to improve plant productivity. However, the intensive and injudicious application of pesticides lead to their accumulation in soils which in turn, may deteriorate the quality of soils besides affecting the agronomically important microbial population of soils (Aamil et al., 2004, Javier Benitez et al., 2006, Nomal, 2006). Such microorganisms often associated with plant growth promoting activities play an important role in the overall performance of crops grown in both conventional and derelict soils. These organisms are however, metabolically inactivated by the excessive application of pesticides to soils (Singh and Wright 2002a, Aamil et al., 2005). Furthermore, the phytotoxic effects of various pesticides on legumes including pea, chickpea, lentil and fababean have been reported (Abbate et al., 2001, Avola et al., 2004, Fox et al., 2007). Severity of these effects however, depends upon the type and concentration of pesticides, the *Rhizobium* species, and plant genotypes (Khan et al., 2004, Wong, 2000, Sawicka and Selwet, 1998). With these considerations, the phytotoxic effects of pre-emergent application of three concentrations [normal (X), double (2X) and three times more of normal (3X)] of technical grade of herbicides (quizalafop-p-ethyl and clodinafop), insecticides (fipronil and pyriproxyfen) and a fungicide (tebuconazole) on popularly grown legume crops like, chickpea, greengram, lentil and pea were evaluated under pot house trials.

A trend of progressive decline was observed for the length of plant organs as the concentration of all pesticides was increased from X to 3X, added to soil. At the

Nitrogen and phosphorus are the major nutrients for plants including legumes. However, the deficiency of these elements in soils leads to severe losses in crop yields. To offset the deficiency of such nutrients, either chemical synthetic fertilizers or biological alternative with phosphate solubilizing activity or nitrogen fixing ability are used in agronomic practices. However, when used under pesticide stress, both the phosphate solubilizing activity and nitrogen fixing potential of bacteria is adversely affected (Wani et al. 2005; Fox et al. 2007). As a result, the overall performance of inoculated plants is negatively affected. The negative impact of pesticides on legumes as observed in this study could probably be due to the disruption of chemical signaling between host plants and N-fixing rhizobia necessary for efficient symbiotic nitrogen fixation and optimal plant yield. By mimicking and disrupting natural phytochemical signaling between legumes and rhizobia, the pesticides used in this study might have significantly delayed the specific timing and initiation of symbiotic signaling crucial for effective nitrogen fixation. Pesticides may also disrupt symbiosis by altering the array of flavonoid phytochemicals, a plant produces or by reducing the overall flavonoid secretion pattern, thereby disrupting plant–rhizobial signalling. The data presented here also show that the application of some pesticides completely abolished nodulation for example in pea. Furthermore, the long-term agricultural studies have shown that nitrogen fixation by bacteria is markedly lower in legume crops that are treated with N fertilizer and pesticides compared with untreated legumes (Fox et al. 2007). However, there are no reports available on the effect of nitrogen and phosphorus on the pesticidal toxicity to either bacteria or plants.

recommended rate of each pesticide, the decline in root and shoot length was not so great but when the legumes were grown in soils treated with 3X of each pesticide, the length of plant organs decreased dramatically for all legumes. Similar inhibitory effects of pesticides on plant growth have been reported by many authors (Khan et al., 2006a, Fox et al., 2007, Tesfamariam et al., 2009). For examples, the herbicides pendimethalin, fluchloralin, terbutryn and methabenzthiazuron when added to soils at lower rates did not affect the growth and dry matter accumulation in chickpea (Kumar et al., 1988, Pahwa and Prakash, 1992) whereas the higher concentration of pendimethalin and basalin (fluchloralin) significantly reduced the growth and dry matter of chickpea (Pahwa and Prakash, 1992). The toxicity of pesticides to dry biomass production by plant organs (roots and shoots) and total dry matter accumulation in chickpea, pea, lentil and greengram plants grown in sandy clay loam soil consistently decreased with increasing concentration of pesticides applied separately. In general, the most toxic effect on dry biomass production was displayed by quizalafop-p-ethyl while clodinafop on the contrary, had little effect on dry biomass production. The adverse effect of insecticides fipronil and pyriproxyfen was comparable to each other. The impact of fungicide, tebuconazole, on dry matter accumulation was rather less pronounced than that observed for quizalafop-p-ethyl. Interestingly, when pesticide tolerant and plant growth promoting bioinoculants (*Mesorhizobium* strain MRC4 for chickpea, *Rhizobium* strain MRP1 for pea, *Rhizobium* strain MRL3 for lentil and *Bradyrhizobium* strain MRM6 and *Pseudomonas aeruginosa* strain PS1 for greengram) were also used along with varying concentrations of herbicides (quizalafop-p-ethyl and clodinafop), insecticides (fipronil and pyriproxyfen) and fungicide (tebuconazole), a major increment in plant biomass of all legumes was recorded when the measured parameters of the uninoculated and inoculated plants grown with the same concentration of each pesticide were compared. Obviously, plant growth promoting rhizobacteria despite reducing pesticidal impact, substantially enhanced the dry mass of chickpea, pea, lentil and greengram plants. The reduction in growth of legume plants following pesticidal application as observed in this study could be due to the toxic effects of pesticides on plant organs, especially the function of nodules which consequently disrupts the legume-*Rhizobium* symbiosis and hence, the N_2 fixation and in turn overall plant growth (Evans et al., 1991). A similar adverse effect of fungicide (thiram) on the performance of chickpea is reported (Gaind et al., 2007). In addition, it is reported that application of increased concentration of herbicides affect the photosynthetic pigments and photo-system II electron transport and therefore, the process of photosynthesis which in turn inversely affect the vegetative growth and seed yield of crops (Eilers et al., 1992, Grossman et al., 2000,

Khan et al., 2006a). The nodulation of chickpea, pea, lentil and greengram plants through their host specific rhizobial partner is an important aspect of legume-*Rhizobium* symbiosis that provides N to the legume plants. The effect of pesticides on symbiosis studied here varied greatly with the types and concentrations of pesticide and age of the plants. Generally, the nodulation on these legume plants decreased with the age of plants, grown either in the absence or presence of pesticides.

Furthermore, the variable nodulation was observed in response to the three concentrations of quizalafop-p-ethyl, clodinafop, fipronil, pyriproxyfen and tebuconazole on root systems of chickpea, pea, lentil and greengram. Each pesticide invariably, decreased the nodule numbers and nodule dry mass when concentration of pesticide was increased from recommended dose to the highest tested rate (3X) applied in this study. Like plant dry matter accumulation, clodinafop had the least inhibitory effect on nodulation while greater damaging effect was observed for quizalafop-p-ethyl. A similar trend for symbiotic properties was observed when bioinoculants specific to each legume crop was added to soils along with pesticides. Moreover, the bioinoculants significantly increased the nodule numbers on the root systems of each crop when compared to uninoculated plants at the same pesticide concentration. Numerous studies have shown that herbicides may inhibit nodulation (Eberbach and Douglas, 1989, Mårtensson and Nilsson, 1989, Isoi and Yoshida, 1990) and N_2 fixation (Mårtensson, 1992, Koopman et al., 1995). For example, Mårtensson (1992) studied bentazon (photosynthetic inhibitor), chlorosulfuron (ALS/AHAS inhibitor) and MCPA (IAA mimic) using Jensen's N-free media to grow red clover (*Trifolium pretense* L.cv. Britta), lucerne (*Medicago sativa* L.cv. Vertus) and birdsfoot-trefoil (*Lotus corniculatus* L.). These herbicides triggered growth disorders such as, root hair deformations that inhibited symbiosis and resulted in fewer nodules. The reason for the inhibitory effect was that these chemicals inhibit photosynthesis and acetolactate synthesis, both of which are important for N_2 fixation. However, the low number of nodules associated with chlorosulfuron application was attributed to an impedance of nodule formation and not nodule initiation (low nodule weights point to an effect on nodule development or maintenance). In yet other report, Anderson et al. (2004) claimed that herbicides may negatively affect the legume-*Rhizobium* relationship by: (i) directly affecting root and shoot biomass of the host plant thereby limiting the number of available sites for rhizobia to attach to or by decreasing the carbohydrate supply to existing nodules (ii) directly affecting rhizobial survival or growth that leads to a decreased potential for rhizobial infection on root hairs (iii) inhibiting or inactivating the biochemical signaling that plants require to initiate nodule development – this inhibition

could affect either rhizobia or plants and (iv) inhibiting nodule development by reducing the capacity for cell division. In other study, Mallik and Tesfai (1985) tested the relative compatibility of selected pesticides at two levels of application (recommended rate and 5× or 10×) with soybean-rhizobia symbiosis in pot culture experiments using a prepared peat inoculant. PCNB, carboxin and carboxin+captan at recommended level were innocuous to growth, nodulation, N_2-fixation and total N content of shoot while carboxin and carboxin+captan at 10 times recommended level proved detrimental to nodulation and N_2-fixation. In addition, carbaryl and malathion at recommended level had no adverse effect while at 10 times recommended level severely reduced N_2-fixation. However, acephate, diazinon and toxaphene at both levels reduced N_2-fixation and total N content. All five herbicides used at recommended and five times recommended level adversely affected nodulation and N_2-fixation. Glyphosate proved least toxic to all parameters at recommended rate, 2,4-DB was less harmful to nodulation and N_2-fixation than trifluralin, alachlor and metribuzin. In case of insecticides, Evans et al. (1993) found that the effectiveness of inoculation with *Rhizobium meliloti* was significantly reduced when inoculant was applied to seed pre-treated with insecticide omethoate. Generally the nodule numbers and shoot mass per plant were reduced by 6 and 22%, compared to plants having no omethoate treatment. In another study, the nodulation in greengram was found to be severely inhibited in the imidachloprid (chloronicotinyl insecticide) treated field compared to control and this inhibitory effect was irretrievable (Kaur and Kaur, 2005). Such inhibitory effect following insecticides application may possibly be due to the inhibition of enzymes involved in growth and metabolisms of plants (Boldt and Jacobsen, 1998). Furthermore, Hashem et al., (1997) reported that fungicides vitavax and benomyl inhibited viability and survival of *Bradyrhizobium* on peanut seeds and in turn decreased *Bradyrhizobium*-peanut symbiosis, nitrogen fixation, plant growth and seed yield. Similarly, inhibitory effects of fungicides crown, arrest and captan on chickpea–*Rhizobium* symbiosis has been reported by Kyei-Boahen et al. (2001). Reports on the effect of pesticides on effective symbiosis of rhizobia with the legume host plants are, however, contradictory. For example, sethoxydim, alachlor, fluazifop butyl and metolachlor at recommended rates did not result in detrimental effects on seed yields or N_2 fixation in soybean while paraquat significantly reduced the amount of N_2 fixed as measured by ^{15}N dilution methods (Kucey et al., 1988). Similarly, the adverse effects of terbutryn/terbuthylazine and bentazone on the performance of pea (Singh and Wright, 2002b) and the phytotoxic effects of chlorimuron-ethyl on *Bradyrhizobium japonicum* inoculated soybean (Zawoznik et al., 2005) is reported. In a similar study, it is reported that

the molecular mechanism of symbiotic inhibition by pesticides is through disruption of signaling between legume (host) plant- derived phytochemicals (luteolin, apigenin) and *Rhizobium* Nod D receptors that is necessary for initiation of nodulation and symbiotic nitrogen fixation (Fox et al., 2007). However, plant growth promoting rhizobacteria including symbiotic N_2 fixers can affect plant development either indirectly by circumventing the toxic effects of pesticides or directly by synthesizing the plant growth regulating substances like phytohormones, siderophores, HCN and ammonia (Jeon et al., 2003, Lopez et al., 2005, Figueiredo et al., 2007, Wani et al., 2008, Yang and Lee, 2008).

Chlorophyll is the most important photosynthetic pigment in plant leaves which plays an important role in converting light energy into chemical energy. Chlorophyll molecule has a cyclic tetrapyrrolic structure (porpyrin) with an isocyclic ring containing a magnesium atom at its centre and a phytol chain attached to it. In our study, all concentrations of quizalafop-p-ethyl, clodinafop, fipronil, pyriproxyfen and tebuconazole decreased chlorophyll (Chl) and leghaemoglobin (Lb) contents measured at pod fill stage (90 DAS) of chickpea, pea (90 DAS), greengram (50 DAS) and lentil (90 DAS) plants, with increasing rates of each pesticide either in the presence or absence of inoculant (*Mesorhizobium* strain MRC4 for chickpea, *Rhizobium* strain MRP1 for pea, *Rhizobium* strain MRL3 for lentil and *Bradyrhizobium* strain MRM6 and *Pseudomonas aeruginosa* strain PS1 for greengram). However, a substantial increase in both Chl and Lb contents was recorded when uninoculated plants were compared with the inoculated ones at the same concentration of each pesticide added to soils. As reported by Boldt and Jacobsen (1998) that the pesticides adversely affect the metabolic enzymes, therefore, it seems probable that pesticides employed in this study might have inversely inhibited the functioning of the enzymes of photosynthetic carbon reduction (PCR) cycle, such as Rubisco, 3-PGA kinase, NADP, NAD-Glyceraldehyde-3-P-dehydrogenase and aldolase. Furthermore, the phytotoxicity of nine pesticides (paraquat, fluazifop-*p*-butyl, haloxyfop, flusilazole, cuproxat, cyazofamid, imidacloprid, chlorpyrifos, and abamectin) at normal dosages on photosynthesis was investigated in vegetable crops like, cucumber (*Cucumis sativus*) as reported by Xia et al. (2006). They observed that plants treated with paraquat had the severest phytotoxic symptoms with the highest reduction in net photosynthetic rate accompanied by declines both in stomatal conductance and intercellular CO_2 concentration. Moreover, Paraquat almost completely inhibited the maximal quantum efficiency of PSII, while other pesticides had no significant effect on quantum efficiency of PSII. In yet another study, Khan et al. (2006b) also reported a similar observation where chlorophyll contents per plant of chickpea were found to decrease consistently with

increasing concentration of linuron, methabenzthiazuron, terbutryn. In contrast, when rhizobacterial strains like, MRC4, MRP1, MRL3, MRL6 and PS1 were also applied with varying concentration of herbicides, insecticides and fungicides, the inoculants not only prevented the decline in chlorophyll and leghaemoglobin contents of chickpea, pea, lentil and greengram but also improved the photosynthetic and symbiotic pigments. The improvement in chlorophyll content in fresh leaves of inoculated legume plants as observed in this study, compared to the uninoculated plants is in agreement to the findings of Elkoca (2008) who also observed a similar increase in chlorophyll content of chickpea foliage with *Rhizobium* and *Bacillus subtilis* and P-solubilizing *Bacillus megaterium*.

Leghaemoglobin, an essential pigment involved in *Rhizobium*-legume symbiosis, play a very critical role in fixing atmospheric N_2 and making it available to developing plants. It is an established fact that the reduction of N_2 to ammonia is catalyzed by the enzyme nitrogenase which is highly sensitive to oxygen. To help protect the nitrogenase, a protein called leghaemoglobin, which binds to oxygen and helps maintain microaerobic conditions within the mature nodule, is produced by legumes following rhizobial infection. This protein is similar in structure to myo- and haemoglobins found in animals; however, it has a higher affinity for oxygen. Interestingly, the protein moiety is encoded by plant genes whereas the haem group is the product of the bacterial genes. In the present study, like Chl, Lb content in fresh nodules collected from the legumes grown in pesticide treated soils, consistently decreased. Of all the tested pesticides, quizalafop-p-ethyl at all the three concentrations decreased significantly the Lb content of all the four legume crops. Bioinoculants on the other hand, influenced the production of leghaemoglobin considerably and increased the Lb content many folds in chickpea, pea, lentil and greengram nodules compared to the uninoculated plants treated with same concentration of pesticides. In a similar study, glyphosate inhibited nodulation and nodule leghaemoglobin content of glyphosate-resistant (GR) soybean. Though, glyphosate accumulated in nodules of field-grown GR soybean, yet its effect on nitrogenase activity of GR soybean was inconsistent in field trials. In greenhouse studies, nitrogenase activity of GR soybean following glyphosate application was transiently inhibited especially in early growth stages, with the greatest inhibition occurring under moisture stress. Studies using bacteroid preparations showed that the level of glyphosate inhibition of bacteroid nitrogenase activity was related to *in vitro* glyphosate sensitivity of the strains (Zablotowicz and Reddy, 2004).

Nitrogen content of the legume plants is one of the most important aspects of *Rhizobium*-legume symbiosis. The nitrogen content in roots and shoots determined at

different stages of chickpea, lentil, pea and greengram differed among treatments. Customarily, the nitrogen content in legume organs decreased with age of plants and was influenced by pesticide concentrations. Among the pesticides, quizalafop-p-ethyl had the largest toxic effect on N content of roots and shoots of chickpea, pea, lentil and greengram. The decrease in N contents of inoculated legume plants might have been due to the reduction in legume- *Rhizobium* symbiosis, as indicated by a decline in the nodulation in this study. In agreement to this findings, Fox et al. (2007) concluded that agrochemicals including insecticides (pentachlorophenol, bisphenol A, DDT and methyl parathion) induce a symbiotic phenotype that inhibits or delays recruitment of rhizobia to host plant roots, fewer root nodules produced, lower rates of nitrogenase activity which in turn reduced N content and a reduction in overall plant yields at harvest. A similar reduction in N uptake in chickpea plants following herbicides application has been reported (Khan et al., 2006a). Moreover, the increased N content of roots and shoots in response to pesticide tolerant bioinoculants is in agreement with Lennox and Alexander (1981) who also observed that N content of beans (*Phaseolus vulgaris*) derived from seeds inoculated with a thiram resistant strain of *Rhizobium phaseoli* were increased following pre-emergent application of thiram.

Also, in the present investigation, similar to N nutrition, P content in roots and shoots of chickpea, pea, lentil and greengram plants estimated at the maturity (harvest) of each crop, decreased progressively with increase in the concentration of each pesticide when legume seeds were treated with and without pesticide tolerant bioinoculants such as, *Mesorhizobium* strain MRC4 (for chickpea), *Rhizobium* strain MRP1 (for pea), *Rhizobium* strain MRL3 (for lentil) and *Bradyrhizobium* strain MRM6 and *Pseudomonas aeruginosa* strain PS1 (for greengram) and grown in sandy clay loam soils. However, bioinoculants in general, increased the P content both in roots and shoots in chickpea, pea, lentil and greengram at all concentration of pesticides when inoculated plants were compared with uninoculated plants grown with the same concentration of pesticides. Generally, P content in both organs of plant though decreased at harvest for each tested crop; the reduction in P content was greater in uninoculated legumes compared to inoculated legume plants. The decreased P content in legume crops observed in this study is however, absolutely contradictory to those reported by Das et al. (2003a) and Das and Mukherjee (2000) who observed that oxyfluorfen, BHC, phorate, carbofuran, and fenvalerate application stimulates microbial populations including phosphate solubilizing bacteria and increases the P pool of soil. However, in this study, the pesticide tolerant strains in general, increased P content suggesting that the tolerant strains might have reduced the uptake of pesticides by plant organs. Further, the P content was found

maximum in both roots and shoots of greengram plants inoculated with the pesticide tolerant and phosphate solubilizing bacterium *Pseudomonas aeruginosa* strain PS1 compared to those observed for *Mesorhizobium* strain MRC4 inoculated chickpea, *Rhizobium* strain MRP1 inoculated pea, *Rhizobium* strain MRL3 inoculated lentil and *Bradyrhizobium* strain MRM6 inoculated greengram plants at all concentrations of herbicides, insecticides and fungicides. Moreover, in the presence of the pesticides at three times of recommended rates, the order of the percent increase in P content of plant organs of greengram inoculated with *Pseudomonas aeruginosa* strain PS1 was found as: quizalafop-p-ethyl>pyriproxyfen>fipronil> tebuconazole>clodinafop for roots and quizalafop-p-ethyl>tebuconazole>clodinafop> pyriproxyfen>fipronil for shoots. Since, the phosphate solubilizing bacteria-plant interaction leads to rendering the unavailable P sources available to the metabolically viable and actively growing plants; hence more P content in roots and shoots of *Pseudomonas aeruginosa* PS1 inoculated greengram plants was obvious in this study. Besides providing P to the plants, the phosphate solubilizing bacteria also augment the growth of plants by stimulating the efficiency of biological nitrogen fixation, enhancing the availability of other trace elements (such as iron and zinc) and by synthesizing phytohormones (Khan et al., 2009a). Hence, overall performance of greengram plants inoculated with phosphate solubilizing bacterium *Pseudomonas aeruginosa* strain PS1 was better than those observed for *Bradyrhizobium* strain MRM6 inoculated plants.

Seed yield and quality of legume grains are the important parameters, which were negatively affected by the pesticides exposure. Generally, the toxicity of agrochemicals on seed yield and grain protein of the four legumes declined consistently with increase in the concentration of herbicides, insecticides and fungicides. Though, seed yield and grain protein of inoculated legumes decreased considerably at the highest tested dose of each pesticide but seed yield and grain protein were even higher than the uninoculated but treated control. In a similar study, a significant improvement in protein content and yield components of legume crops following inoculation with plant growth promoting bacteria is reported (Wani et al., 2007a, Rinu and Pandey, 2009, Radha et al., 2009). However, the reduction in seed attributes following herbicides, insecticides and fungicidal application could probably, be due to inhibition of the enzymes and functional proteins of metabolic pathways involved in protein synthesis (Boldt and Jacobsen, 1998). Additionally, since all bioinoculants used in this study were pesticide tolerant that grew well on minimal media devoid of N and C sources and produced plant growth promoting substances, therefore, there is reason to believe that these bioinoculants might have used some fraction of pesticides

added to soils as N or C sources thereby reducing the degree of toxicity of such agrochemicals to plants under study. As a consequence of these activities, seed yield and grain protein of inoculated legumes when grown even in the pesticide stressed soils, increased substantially.

A comparable report on the effect of pesticides on legumes has been reported. For example, different concentrations (e.g. 5, 10 and 25 ppm) of insecticides aldicarb, carbofuran, phorate fensulfothion and fenamiphos were tested for their pesticidal impact on chickpea plants by Tiyagi et al. (2004). Significant improvement in plant growth was noted at lower concentrations (5 and 10 ppm) of different pesticides but 5 ppm concentration proved highly effective and non-phytotoxic. However, the phytotoxic effect on chickpea plants was more pronounced with 25 ppm concentration of all pesticides. Generally, the efficient fungicides have been the most damaging to *Rhizobium* (Aggarwal, 1986). However, some fungicides, like captan and carbendazim did not show any deleterious effect on native *Rhizobium* colonization suggesting that such fungicides were compatible with nodule bacteria. As a consequence when inoculated, *B. japonicum* increased the nodulation and nitrogen fixation in soybean plants (Kaur et al., 2007). Similarly, the fungicides when applied with *Rhizobium* have shown to enhance nodule formation and therefore biological nitrogen fixation of food grain legumes, like common bean, greengram and lablab (Muthomi et al., 2007). In contrast, Aggarwal (1986) evaluated the effect of carbamate on nodulation and N_2 fixation in *Pisum sativum* and *Vigna sinensis* and reported that the low concentrations of the pesticides had little effect on nodulation and N_2 fixation whereas higher concentrations adversely affected the measured parameters. In a similar study, Alonge (2000) evaluated the phytotoxicity of imazaquin on the growth of soybean plants and found that chlorophyll a and total chlorophyll concentration in fresh foliage, root nodules, shoot growth, whole plant dry weight and grain yield were reduced. In comparison, the plants grown in the presence of bio-inoculant increased the seed yield and seed protein. Moreover, nodulation (nodule number per plant and their dry mass) and chlorophyll contents per plant decreased consistently with increasing concentration of each herbicide, except linuron, which improved nodulation (Khan et al. 2006b). Bentazone, isoproturon, fluchloralin and 2,4-D at higher dose adversely affected vitality, chlorophyll content, N and protein content, nodulation and seed production in chickpea inoculated with *Mesorhizobium ciceri*. Protein content in seeds increased significantly following herbicide applications but decreased with an increase in application rates and ten times the recommended rates of bentazone and 2,4-D completely decreased nodulation (Khan et al. 2004).

The use of pesticides to agronomic soils may affect soil biological activity in a variety of ways. For example, herbicides may have negative effects on the growth of rhizobia (Mårtensson, 1992; Singh and Wright, 2002a) and influence nodulation and biological nitrogen fixation in legumes (Boldt and Jacobsen 1998). However, pesticides applied at lower rates are reported to have no negative impact both on bacteria and plants. For instance, the pesticides, like, cotrazine (atrazine 80W) and northrin ®10EC stimulated the dehydrogenase activity of the microbial community at low concentrations (0.2%) while inhibited it at high concentrations (Nweke et al. 2007). Similarly, respiratory activity in soil was stimulated with the application of 50 mg atrazine/kg of soil (Hu et al., 2005). Such stimulatory effect could be attributed to the use of the pesticides as source of electron and energy. It is obvious from this study that lower dose rates of pesticides showed considerably lower toxicity to both rhizobacteria and legume plants compared to the higher dose of the same pesticides. Thus, application of the pesticides at lower rates as used in this study is not likely to inhibit the metabolic activity of the tested rhizobacteria. Furthermore, in the present study, the sensitivity of different pesticides to plant growth promoting activities of rhizobacterial varied considerably even at lower concentration rates because specific bacterial genera more readily metabolize one pesticide of specific chemical group than other one (Fox et al., 2007). In other study, the changes in aerobic bacteria, autotrophic nitrifiers, respiration and nitrification in soils treated with cinosulfuron at 42 (field rate) and 4200 µg/kg was observed one and four weeks after incubation. Only nitrification was slightly inhibited by the cinosulfuron treatment, even at the field rate. While, the higher rates of cinosulfuron (100 mg/l) negatively affected the growth of aerobic bacteria, and *Azotobacter* strains under conditions similar to those of soil environment (Allievi and Gigliotti, 2001). Similarly, trifluralin at lower concentrations stimulated the growth of *Azotobacter chroococcum* and *Bradyrhizobium japonicum*. Interestingly, not only the populations of these bacteria increased at lower concentrations of trifluralin, but also the size of colonies enlarged and appeared very quickly. In comparison, higher concentrations of trifluralin restricted the formation of microbial colonies and the acetylene reduction activity of *A. chroococcum* suggesting that the microbial communities of soils could utilize trifluralin as sole C and N sources for their growth (Hang et al., 2001). In soil, the lower rates of pesticides are adsorbed or form complexes with organic and inorganic matter, reducing chemical mobility and consequently, reduced toxicity to plants (Nweke et al. 2007).

In this study, when quizalafop-p-ethyl, clodinafop, fipronil, pyriproxyfen, and tebuconazole used alone had a substantial deleterious effect on chickpea, pea, lentil and greengram plants grown in sandy clay loam soils. The inoculation of pesticide tolerant and plant growth promoting *Mesorhizobium* strain MRC4 (for chickpea), *Rhizobium* strain MRP1 (for pea), *Rhizobium* strain MRL3 (for lentil) and *Bradyrhizobium* strain MRM6 and *Pseudomonas aeruginosa* strain PS1 (for greengram) on the other hand, not only protected the chickpea, pea, lentil and greengram plants from pesticidal toxicity and but also increased the growth, symbiosis and yields of test crops through their plant growth promoting activities. The increased growth of inoculated greengram plants even in the presence of pesticides might have possibly been due to the synthesis and release of plant growth promoting substances like Indole acetic acid, siderophores like phenolates (salicylic acid and 2,3 2,3-dihydroxy benzoic acid), exopolysaccharides, hydrogen cyanides and ammonia. More importantly, the essential nutrients required for better performance of legumes under both conventional and derelict soils is P and N which in this study, was made available by the pesticide tolerant phosphate solubilizing bacterium (*Pseudomonas aeruginosa* strain PS1) and nitrogen fixing rhizobia, respectively. Moreover, it has been reported earlier that exopolysaccharides (EPS) are known to influence legume root infection and nodulation (Chen et al., 1985, Leigh et al., 1988). In the present study, different plant growth promoting rhizobacteria applied as bioinoculant for legume crops were able to produce EPS in detectable amounts even in the presence of higher concentrations of herbicides, insecticides and fungicides. Therefore, it is possible that the production of EPS by the selected strains might have improved the symbiosis between inoculated bacterial cultures and their corresponding legume host plants and consequently, the yields of crops. Thus, *Mesorhizobium* strain MRC4, *Rhizobium* strain MRP1, *Rhizobium* strain MRL3, *Bradyrhizobium* strain MRM6 and *Pseudomonas aeruginosa* strain PS1 with multiple plant growth promoting activities could be developed as super bioinoculant for increasing the fertility of soils and consequently the productivity of legume crops even under pesticide enriched soils.

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Appendix

Appendix 1

Nutrient agar (g/l)

Beef extract 3; peptone 5; agar 15; pH 7

Appendix 2

Martin's medium

Dextrose 5; potassium dihydrogen ortho-phosphate 1; magnesium sulphate 0.5; streptomycin 0.006; rose Bengal 2 part in 3000 part of medium.

(1g of chloramphenicol/nalidixic acid was dissolved in 100 ml of sterile water. 0.3 ml of this solution was added to 100 ml of rose Bengal medium after it cooled to 45^0C).

Appendix 3

Pikovskaya medium (g/l)

Glucose 10; $Ca_3(PO_4)_2$ 5; $(NH_4)_2SO_4$ 0.5; NaCl 0.2; $MgSO_4.7H_2O$ 0.1; KCl 0.1; yeast extract 0.5; $MnSO_4$ and $FeSO_4$ trace; pH 7

Appendix 4

Yeast extract mannitol medium (g/l)

Mannitol 10; K_2HPO_4 0.5; $MgSO_4.7H_2O$ 0.2; NaCl 0.1; yeast extract 1.0 ; $CaCO_3$ 2; pH 7

Appendix 5

Gram staining

***Primary stain*: *Solution A*-** Crystal violet (90% dye content) 2 g; Ethyl alcohol (95%) 20 ml, ***Solution B*-** Ammonium oxalate 0.8 g; distilled water 80 ml

Gram's iodine

Iodine 1 g; potassium iodide 2 g; distilled water 300 ml

Decolorizer

Ethyl alcohol 95 ml; distilled water 5 ml

Counter stain

Safranin (2.5% solution in 95% ethyl alcohol) 10 ml; distilled water 100 ml

Appendix 6

Kovac's reagent

p-dimethyl amino benzaldehyde 10 g; Iso-amyl alcohol 15 ml

(Dilute 10 times in distilled water before use)

Appendix 7

MR-VP broth (g/l)

Peptone 7; dextrose 5; potassium phosphate 5; pH 6.9

Appendix 8

Methyl red solution (g/l)

Methyl red 0.1; ethyl alcohol 300 ml; distilled water 200 ml

Appendix 9

Barrit's reagent (g/l)

Solution A

A- naphthol 5; ethanol 95 ml

Solution B

Creatine 0.30; potassium hydroxide 40

Appendix 10

Simmons citrate agar (pH 7.0 ± 0.2) (g/l)

Ammonium dihydrogen phosphate 1; dipotassium phosphate 1; magnesium sulfate 0.2; sodium chloride 5; sodium citrate 2; bromothymol blue 0.08

Appendix 11

Trypticase nitrate broth (g/l)

Trypticase 20; disodium phosphate 2; dextrose 1; potassium nitrate 1; agar 20; Ph 7

Solution A (g/l)

Sulfanilic acid 8; acetic acid 5N 1000 ml

(5N: 1 part glacial acetic acid to 2-5 parts distilled water)

Solution B (g/l)

Dimethyl amine 1- napthylamine 5; acetic acid 1000 ml

Appendix 12

Fermentation broth (g/l)

Beef extract 1; peptone 10; phenol red 0.018; pH 7.4

Appendix 13

Starch agar (g/l)

Peptone 5; beef extract 3; starch 2; agar 20; pH 7.0

Appendix 14

Minimal salt agar medium (g/l)

KH_2PO_4 1; K_2HPO_4 1; NH_4NO_3 1; $MgSO_4.7H_2O$ 0.2; $CaCl_2.2H_2O$ 0.02; $FeSO_4.7H2O$ 0.01; pH 6.5

Appendix 15

Luria Bertani (LB) broth (g/l)

Tryptone 10; yeast extract 5; Nacl 10; pH 7.5

Appendix 16

Chrome Azurol S (CAS) agar medium

CAS agar is prepared from four solutions

Solution 1: Fe-CAS indicator solution

Mix 10 ml of 1 mM $FeCl_3.6H_2O$ [in 10 Mm HCl} with 50 ml of an aqueous solution of CAS (1.21 mg/ml). The above solution was then added to 40 ml of HDTMA (1.82 mg/ml) and cooled to 50 ^{0}C.

Solution 2: Buffer solution

Dissolve 30.24 g of PIPES in 750 ml of a salt solution containing 0.3 g KH_2PO_4, 0.5 g NaCl and 1 g NH_4Cl, pH 6.8 with 50% KOH and water was added to bring the volume to 800 ml.

Solution 3: in 70 ml water

2 g glucose, 2 g mannitol, 493 mg $MgSO_4.7H_2O$, 11 mg $CaCl_2$, 1.17 mg $MnSO_4.H_2O$, 1.4 mg H_3BO_3, 0.04 mg $CuSO_4.5H_2O$, 1.2 mg $ZnSO_4.7H_2O$ and 1 mg $Na_2MoO_4.2H_2O$. Autoclaved , cooled to 50 ^{0}C, then added to the buffer solution along with 30 ml filter-sterilized 10% (W: V) casamino acids (solution 4). The indicator solution was added last with sufficient stirring to mix the ingredients without forming bubbles.

Appendix 17

Modi medium

K_2HPO_4 0.05%; $MgSO_4$ 0.04%; NaCl 0.01%; mannitol 1%; glutamine 0.1%; NH_4NO_3 0.1%

Appendix 18

Chloromolybdic acid

Ammonium molybdate 15 g; distilled water 400 ml; 10 N HCl 400 ml

The above described materials were mixed slowly with rapid stirring, cool and make the volume to 1 liter with distilled water

Appendix 19

Chlorostannous acid

Stannous chloride 10 g; concentrated hydrogen chloride 25 ml

The stock solution was kept in air tight bottle. 1ml of stock solution is mixed in 132 ml of distilled water at the time of experiment.

Appendix 20

HCN induction medium (g/l)

Tryptic soy broth 30; glycine 4.4; agar 15

Appendix 21

Peptone water (g/l)

Peptone 10; NaCl 5; pH 7

Appendix 22

Nessler's reagent

Potassium iodide 50 g; distilled water (ammonia free) 35 ml

Add saturated aqueous solution of mercuric chloride until a slight precipitate persists

Potassium hydroxide 400 ml

Dilute the solution to 1000 ml with ammonia free distilled water. Allow to stand for one week, decant supernatant liquid and store in a tightly capped amber bottle.

Appendix 23

Physical and chemical analysis of soil

Sand	667 g kg^{-1}
Silt	190 g kg^{-1}
Clay	143 g kg^{-1}
pH	7.2
Water holding capacity	0.44 ml g^{-1}
Cation exchange capacity	11.7 cmol kg^{-1}
Organic matter	6.2 g kg^{-1}
Kjeldahl N	0.75 g kg^{-1}
Olsen P	16 mg kg^{-1}
Anion exchange capacity	5.1 cmol kg^{-1}

Appendix 24

Phosphate buffer 1% (pH 7.2-7.4)

Solution A- Disodium phosphate 1.4 g; distilled water 100 ml

Solution B-Sodium dihydrogen phosphate 1.4 g; distilled water 100 ml

(84.1 ml of solution A to 15.9 ml of solution B and 8.5 g of sodium chloride and volume was made upto one liter)

Appendix 25

Pyridine reagent

Sodium hydroxide 0.8 g (dissolved in 50 ml), pyridine 33.8 ml. The volume was made upto 100 ml

Appendix 26

Copper solution

Solution A: Sodium carbonate 2g (mixed with 0.1 N NaOH)

Solution B: Copper sulphate 0.5 g, potassium sodium tartrate 1g, distilled water 100 ml

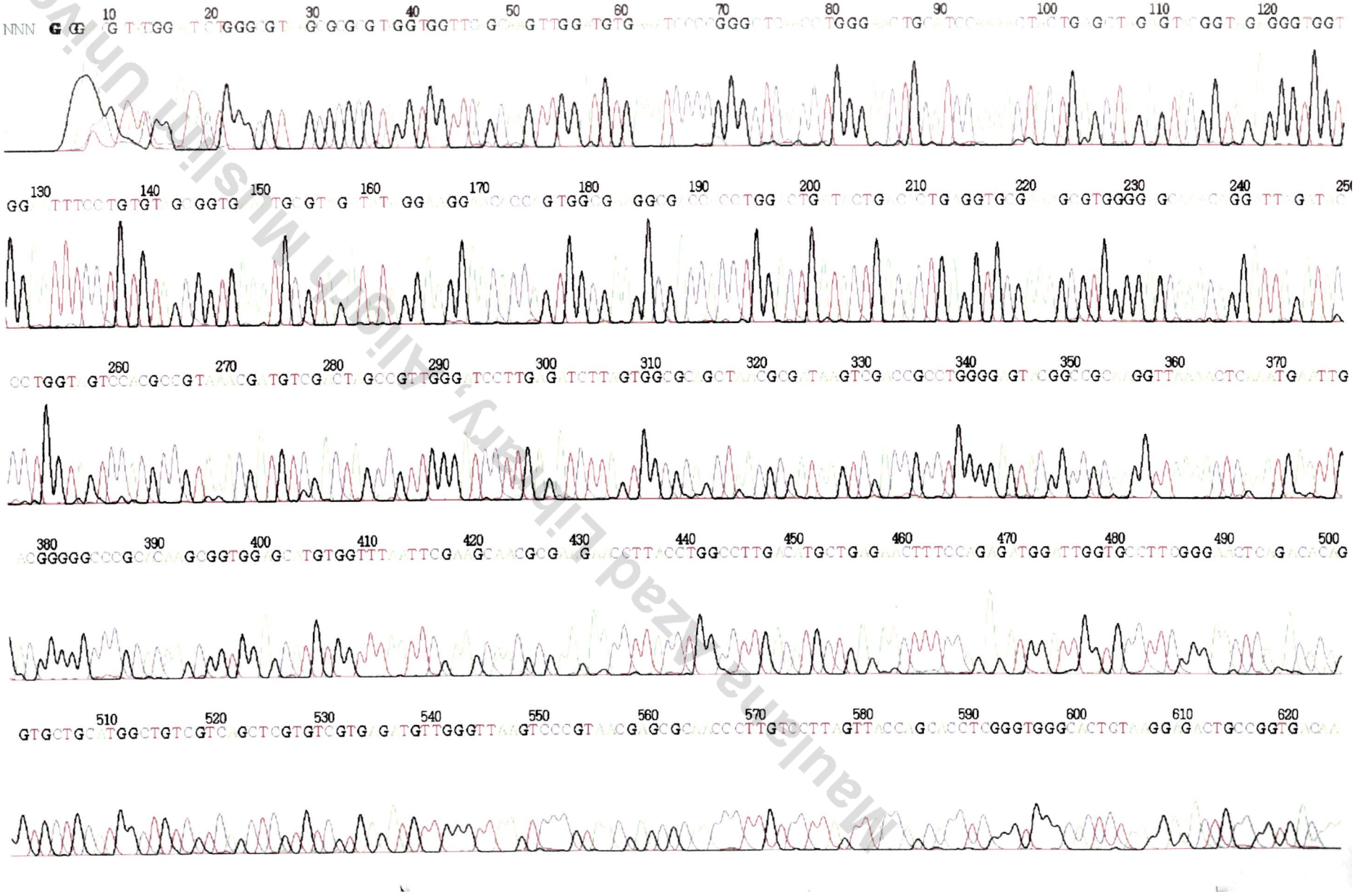
Copper solution was prepared by mixing 50 ml solution A with 1 ml of solution B

Appendix 27

Folins reagent

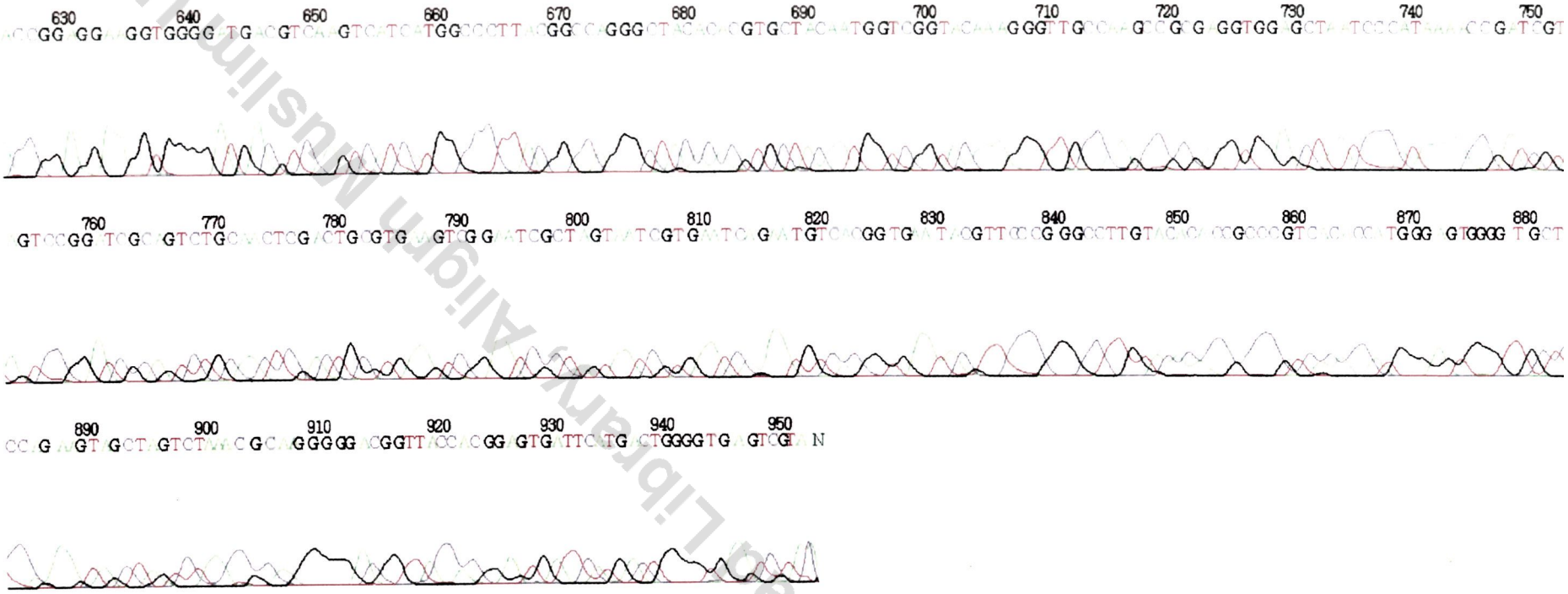
Sodium tungstate 100 g, sodium molybdate 25 g, distilled water 700 ml, 85% ortho-phosphoric acid 50 ml, HCl 100 ml, bromine water few drops (Reflux the above given mixture for 10 h). Boil the solution without condenser for 15 min. to remove excess bromine, cool and dilute it to 1 liter

File: PS1-518F.ab1 Run Ended: 2008/9/20 7:51:58 Signal G:1118 A:1361 C:1544 T:1377
Sample: PS1_518F Lane: 19 Base spacing: 14.03 954 bases in 11279 scans



File: PS1-518F.ab1 *Run Ended: 2008/9/20 7:51:58* *Signal G:1118 A:1361 C:1544 T:1377*

Sample: PS1_518F *Lane: 19* *Base spacing: 14.03* *954 bases in 11279 scans* *Page 2 of 2*



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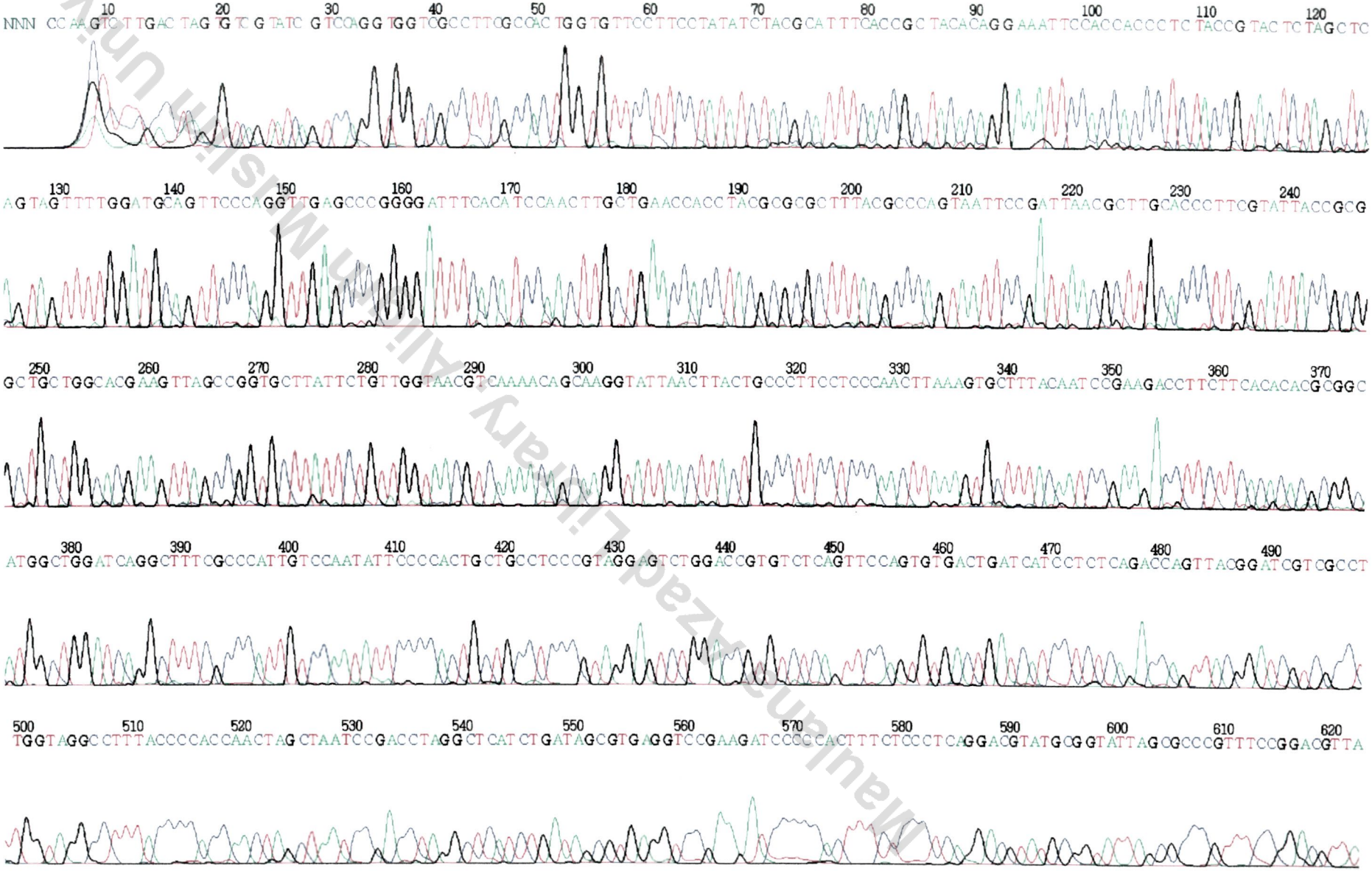
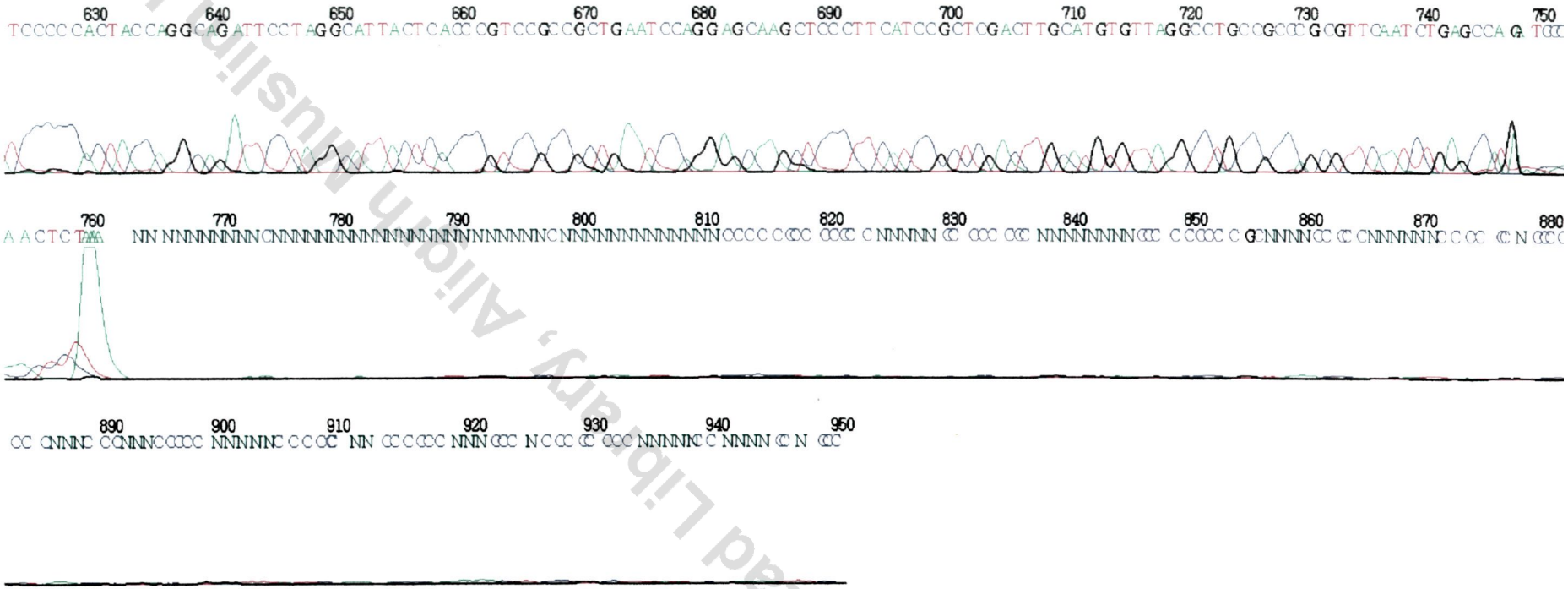


File: PS1-800R.ab1 *Run Ended: 2008/9/20 7:51:58* *Signal G:1092 A:976 C:1504 T:1489*

Sample: PS1_800R *Lane: 17* *Base spacing: 14.03* *950 bases in 11306 scans* *Page 2 of 2*



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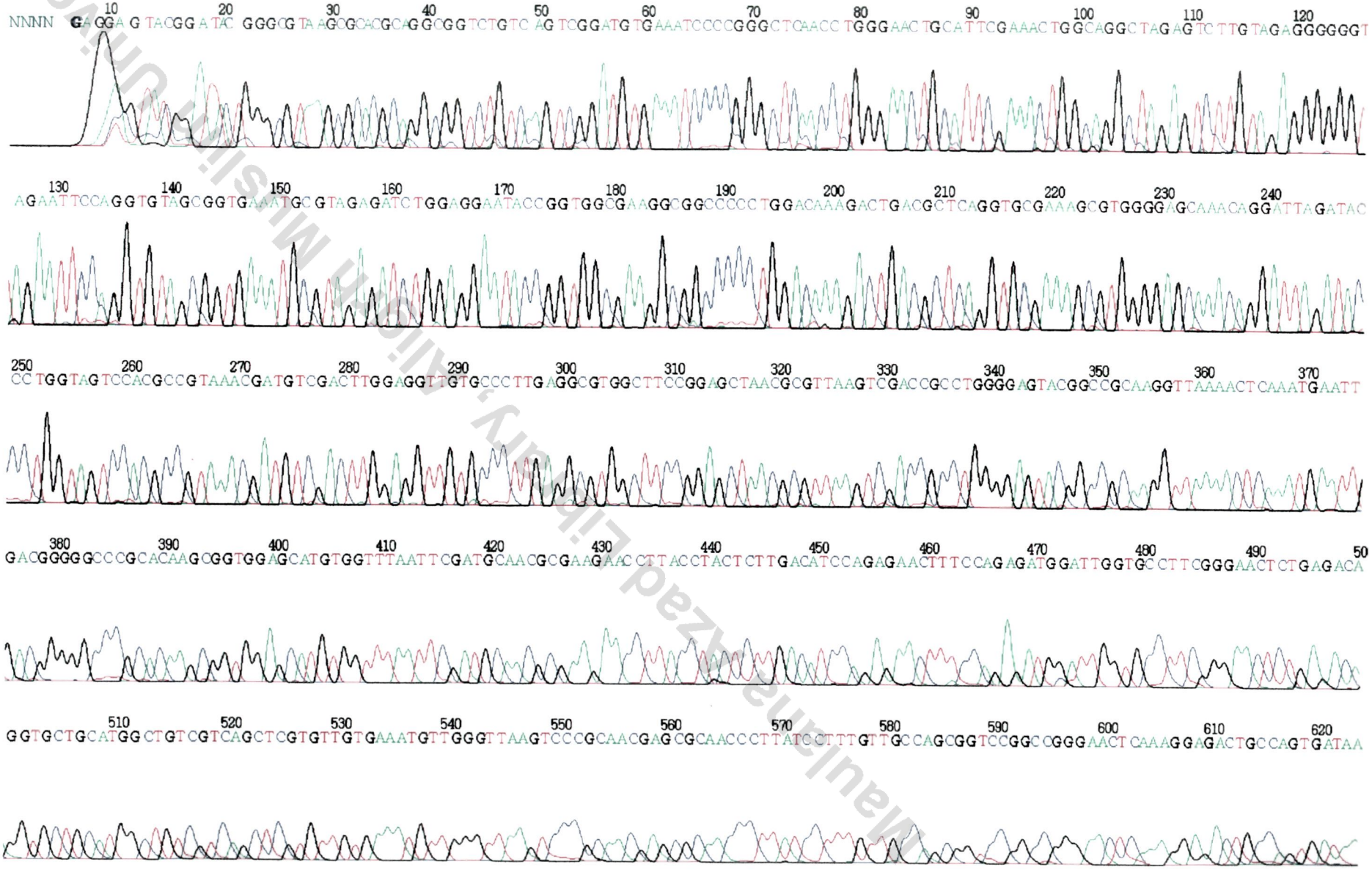
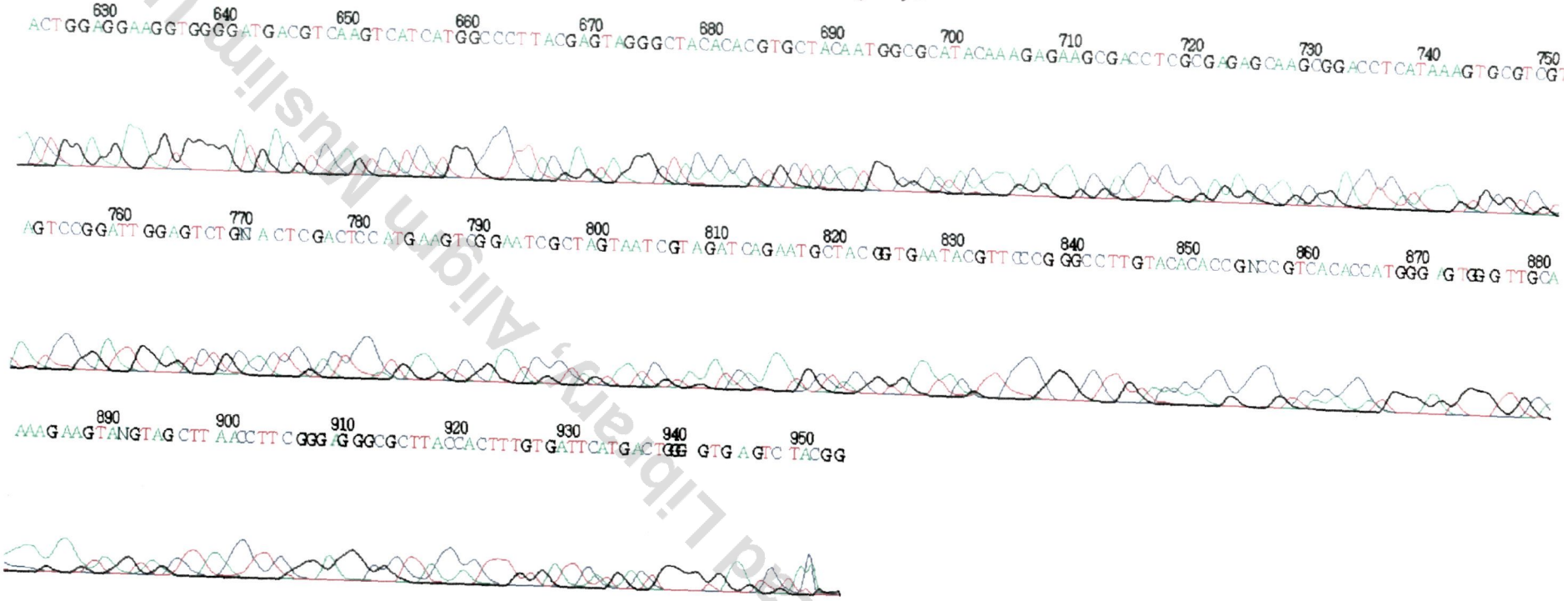
File: PS2-518F.ab1 Run Ended: 2008/9/20 7:51:58 Signal G:1904 A:1886 C:1896 T:1754
Sample: PS2_518F Lane: 32 Base spacing: 13.84 954 bases in 11312 scans

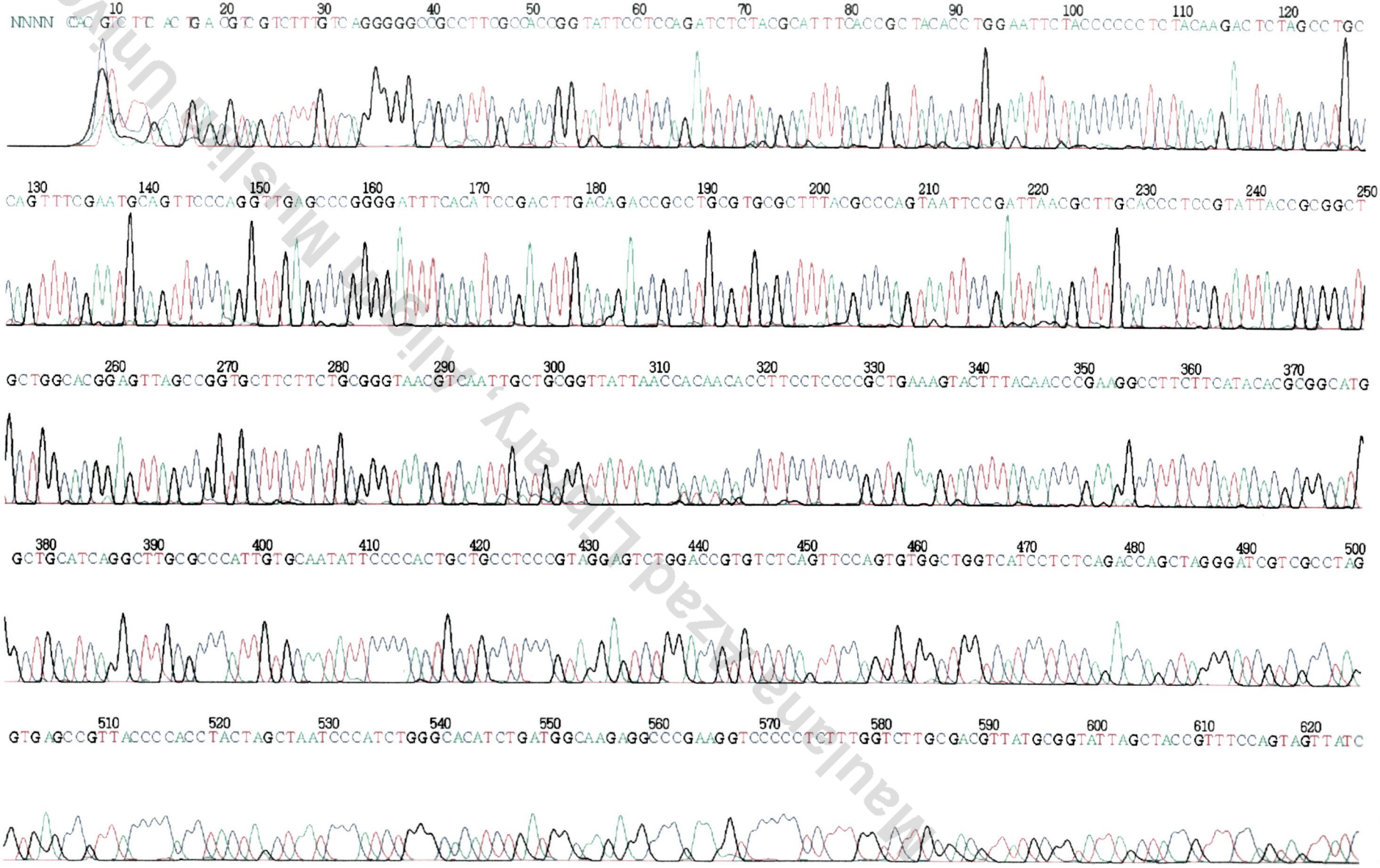


File: PS2-518F.ab1 Run Ended: 2008/9/20 7:51:58 Signal G:1904 A:1886 C:1896 T:1754

Sample: PS2_518F Lane: 32 Base spacing: 13.84 954 bases in 11312 scans Page 2 of 2

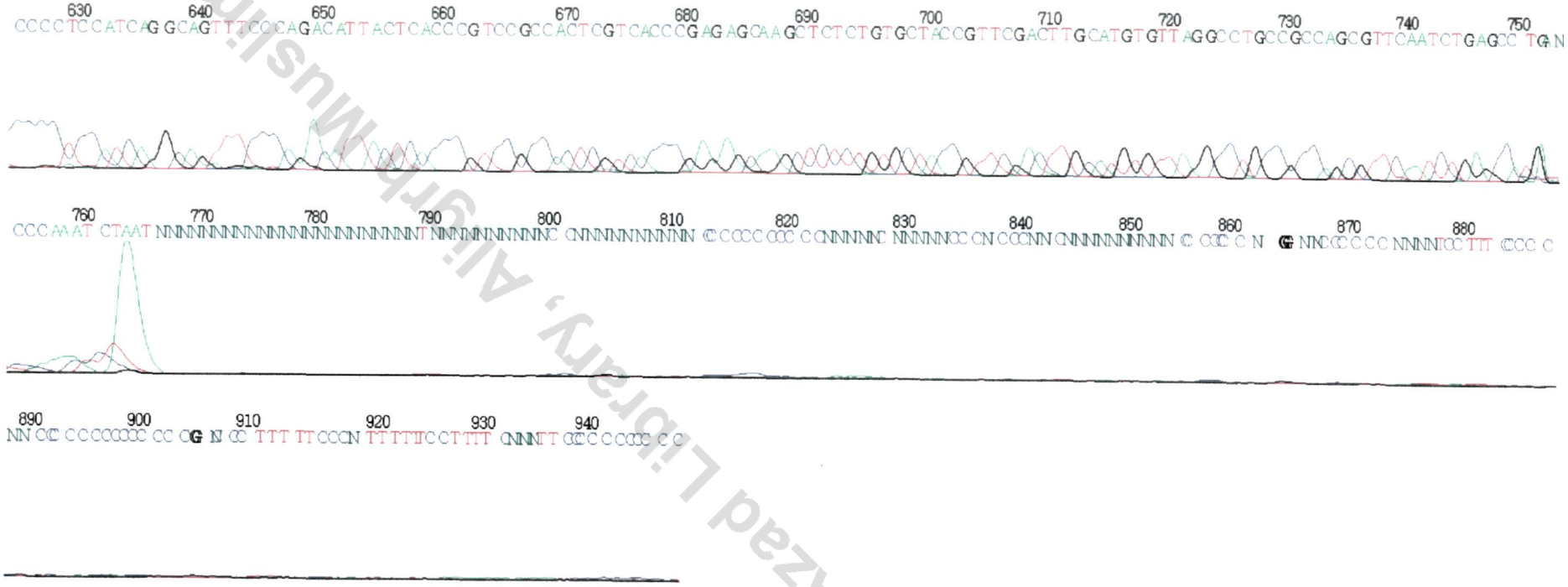


File: PS2-800R.ab1 Run Ended: 2008/9/20 7:51:58 Signal G:945 A:739 C:1578 T:1283
Sample: PS2_800R Lane: 30 Base spacing: 13.65 948 bases in 11151 scans

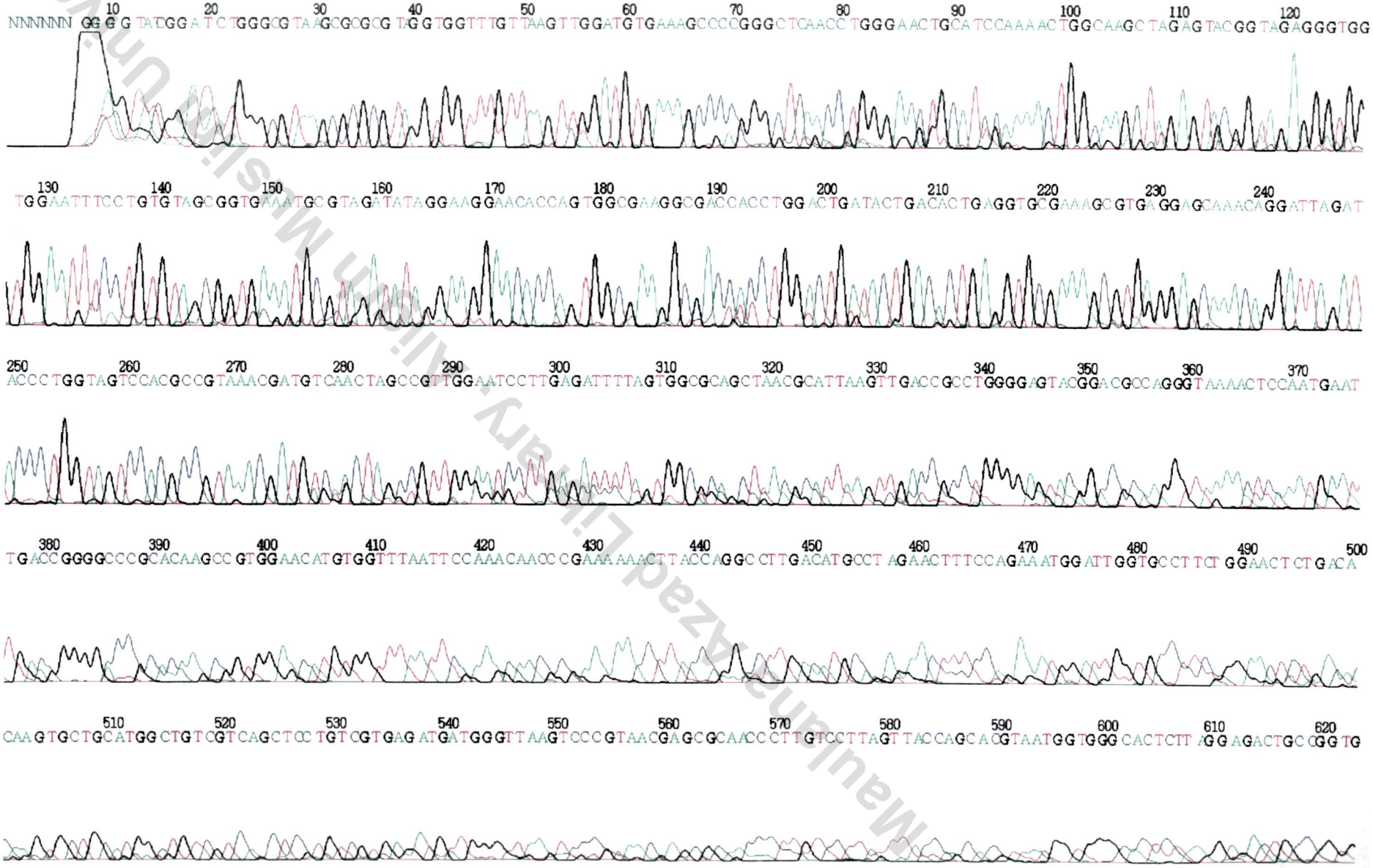


File: PS2-800R.ab1 Run Ended: 2008/9/20 7:51:58 Signal G:945 A:739 C:1578 T:1283

Sample: PS2_800R Lane: 30 Base spacing: 13.65 948 bases in 11151 scans

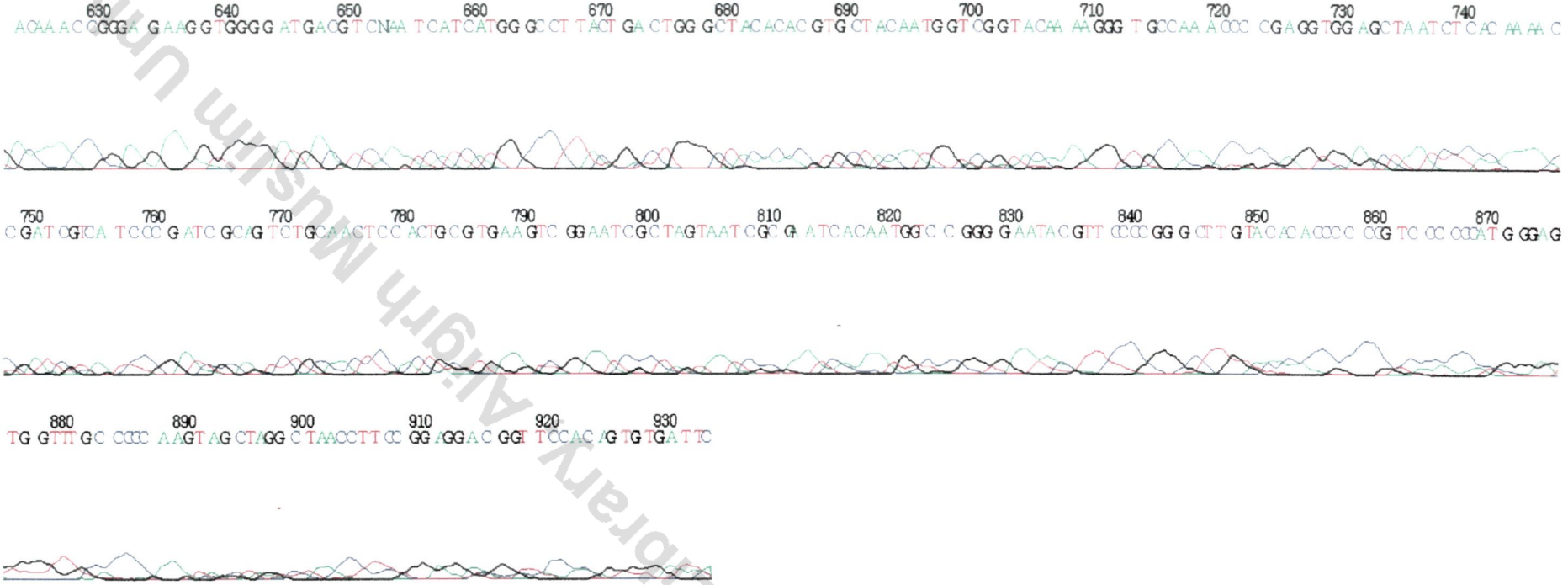


File: PS9-518F.ab1 Run Ended: 2008/9/20 7:51:58 Signal G:626 A:586 C:616 T:564
Sample: PS9_518F Lane: 28 Base spacing: 13.65 934 bases in 11181 scans



File: PS9-518F.ab1 Run Ended: 2008/9/20 7:51:58 Signal G:626 A:586 C:616 T:564

Sample: PS9_518F Lane: 28 Base spacing: 13.65 934 bases in 11181 scans Page 2 of 2



File: PS9-800R.ab1 Run Ended: 2008/9/20 7:51:58 Signal G:1025 A:868 C:1660 T:1317
Sample: PS9_800R Lane: 26 Base spacing: 13.84 946 bases in 11243 scans Page 1 of 2

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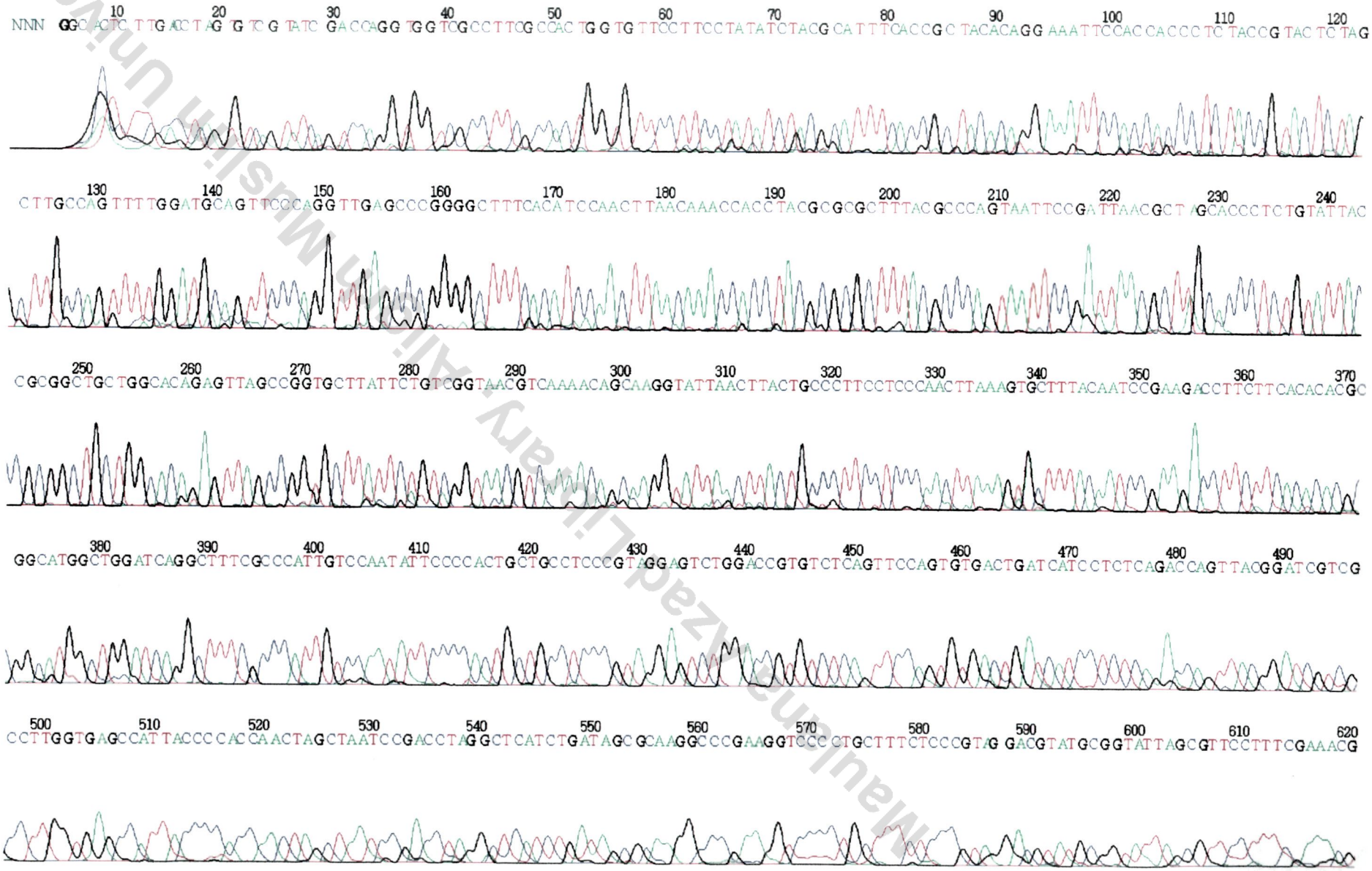
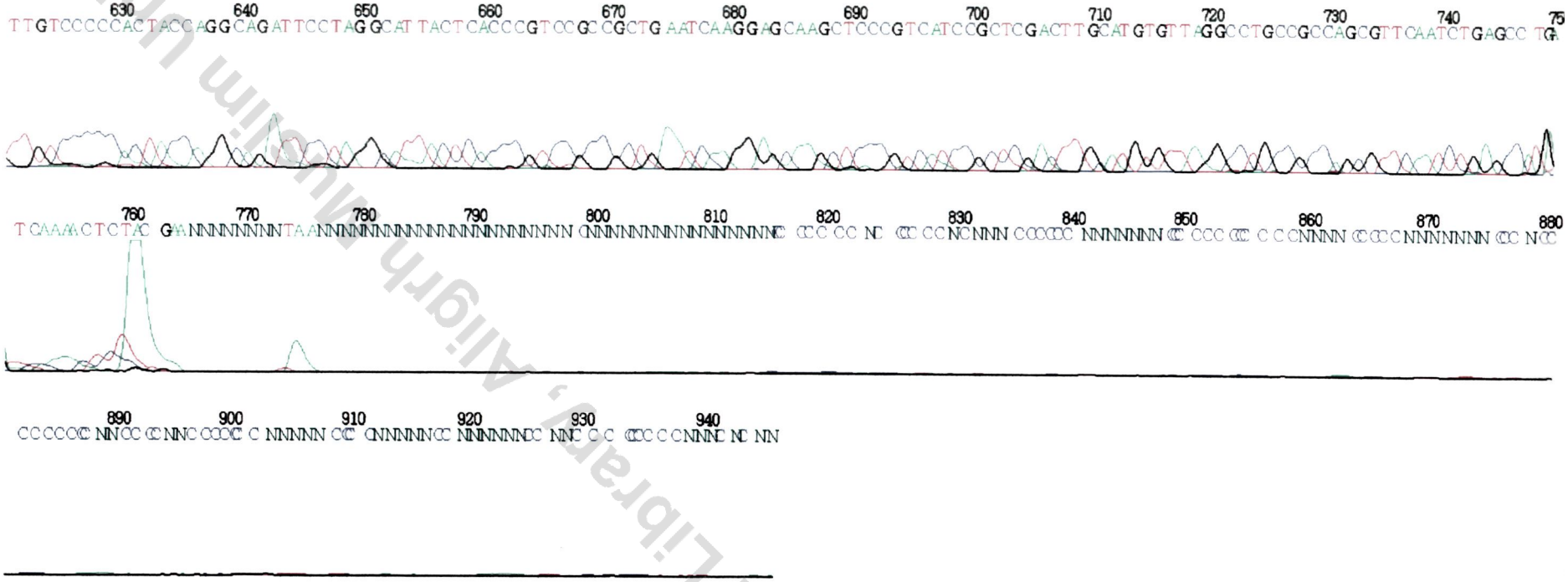


File: PS9-800R.ab1 *Run Ended: 2008/9/20 7:51:58* *Signal G:1025 A:868 C:1660 T:1317*

Sample: PS9_800R *Lane: 26* *Base spacing: 13.84* *946 bases in 11243 scans*



File: PS19-518F.ab1 Run Ended: 2008/9/20 7:51:58 Signal G:1561 A:2135 C:2594 T:2368
Sample: PS19_518F Lane: 24 Base spacing: 13.84 952 bases in 11252 scans Page 1 of 2

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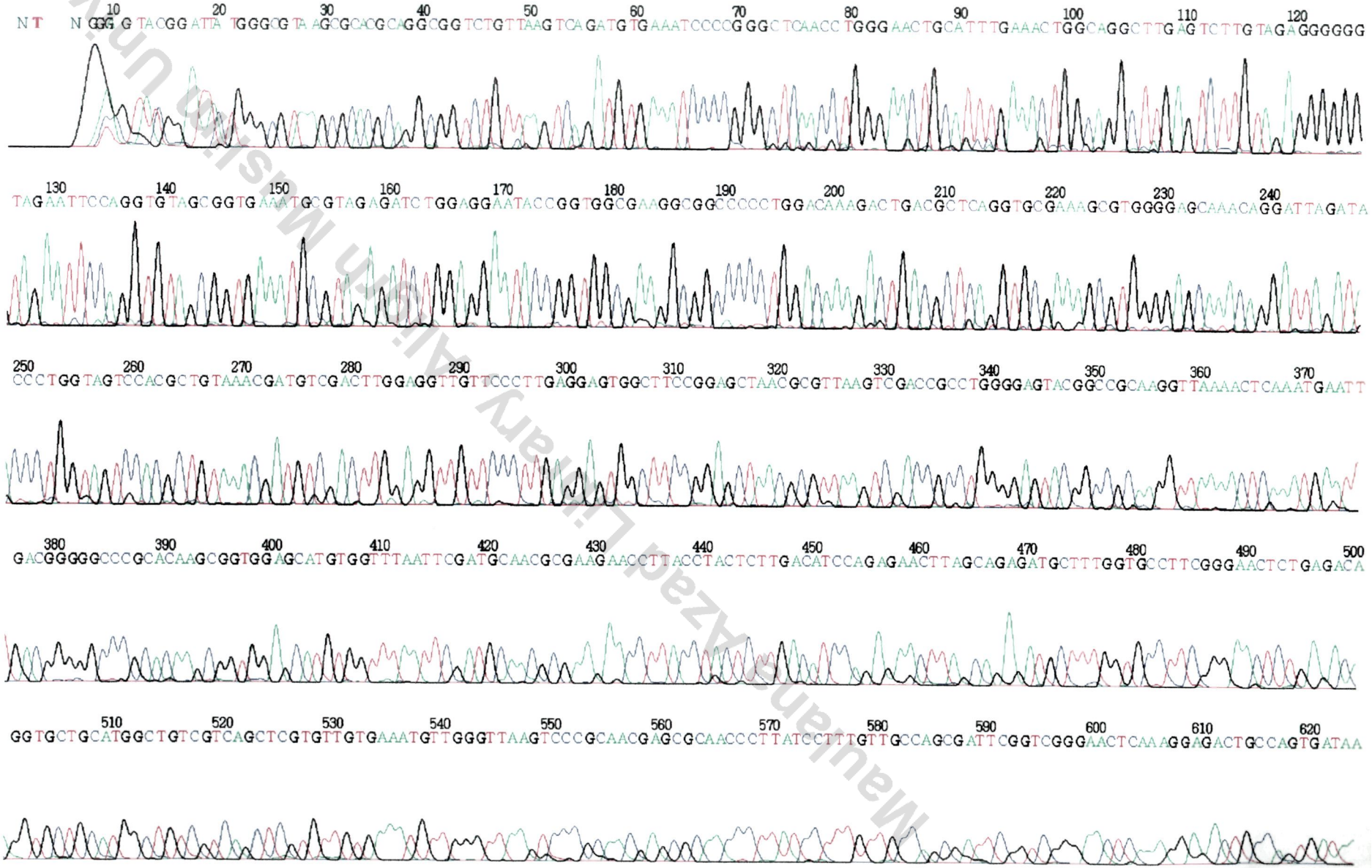


File: PS19-518F.ab1 Run Ended: 2008/9/20 7:51:58 Signal G:1561 A:2135 C:2594 T:2368

Sample: PS19_518F Lane: 24 Base spacing: 13.84 952 bases in 11252 scans Page 2 of 2

630 640 650 660 670 680 690 700 710 720 730 740 750

ACTGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGAGTAGGGCTACACACGTGCTACAATGGCATATACAAAGAGAAGCGACCTCGCGAGAGCAAGCGGACCTCATAAAGTATGTCG

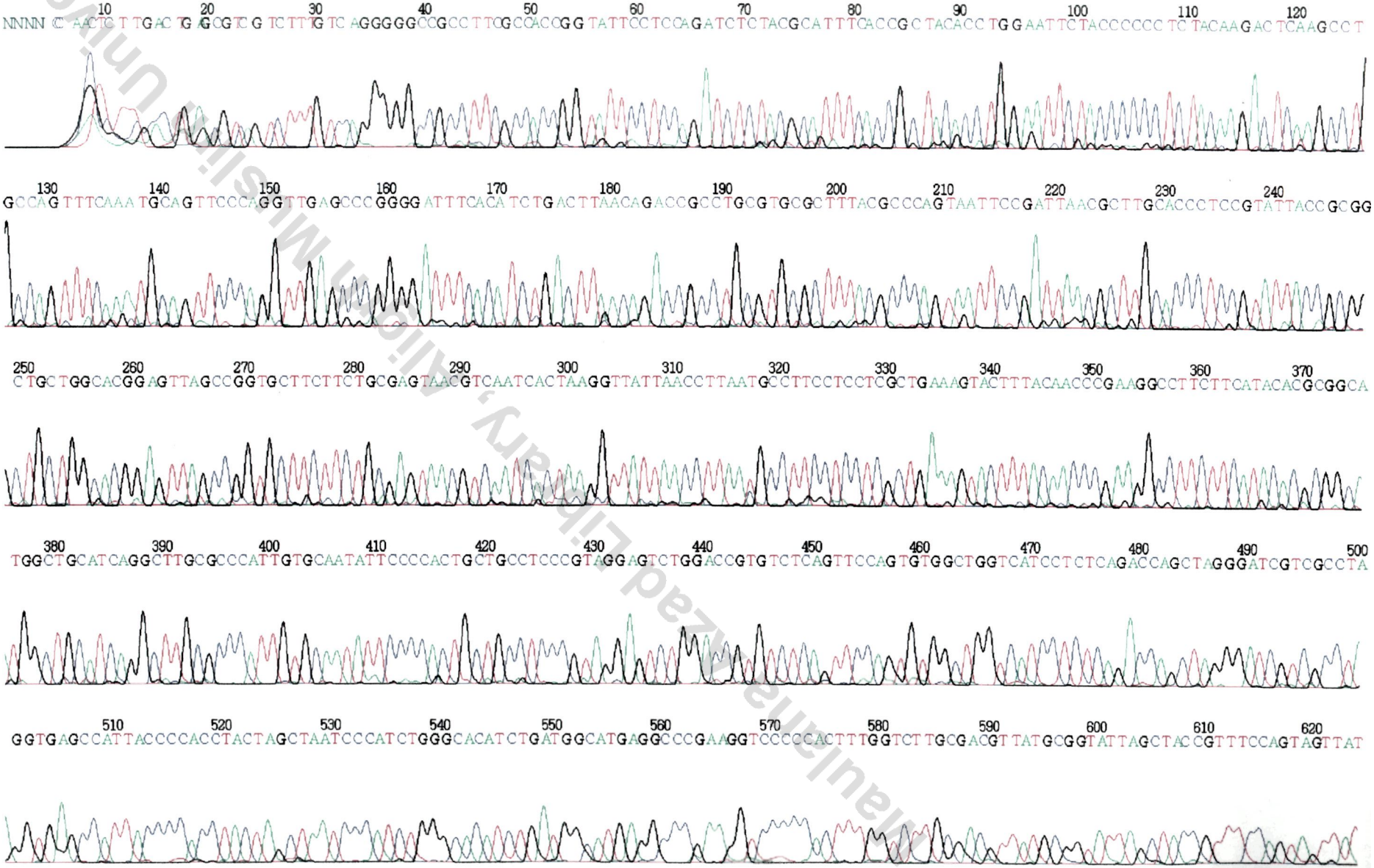
760 770 780 790 800 810 820 830 840 850 860 870 880

TAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTCGGAATCGCTAGTAATCGTAGATCAAATGCTACGGTGAATACGTTCCCGGGCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCAA

890 900 910 920 930 940 950

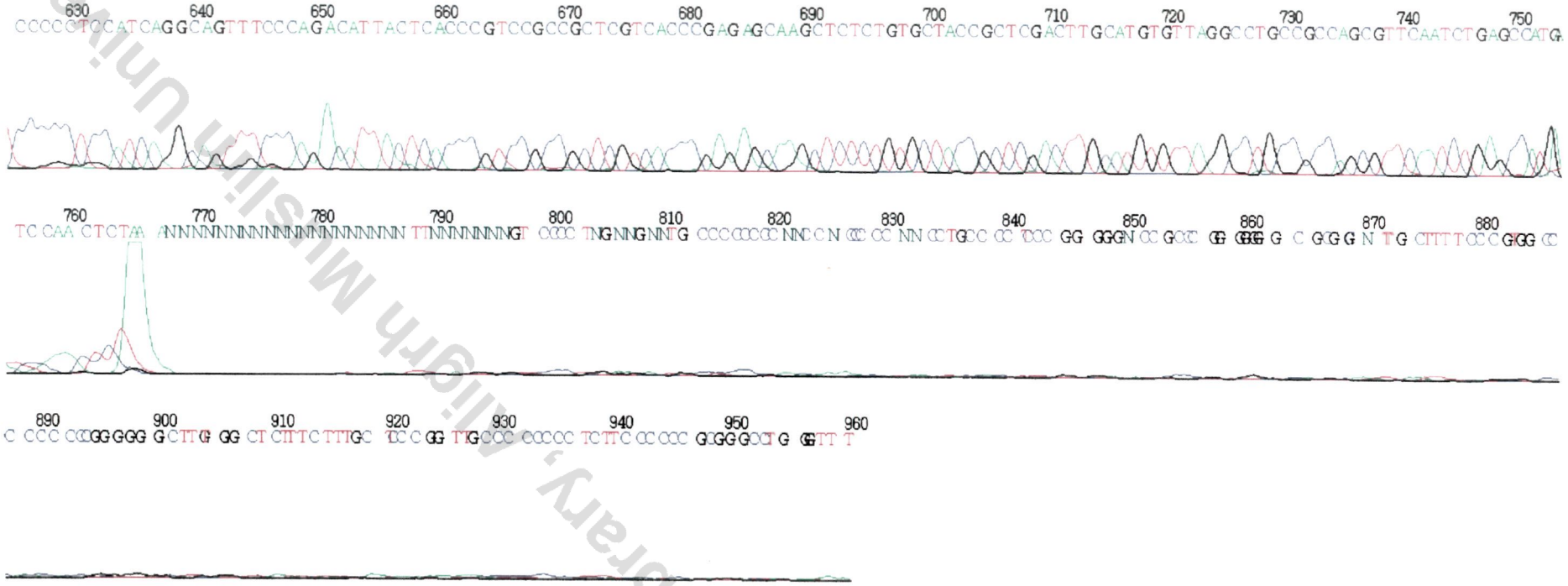
AAGAAGTANGTAGCTTAACCTTCGGGAGGGCGCTTACCACTTTGTGATTCATGACTGGGGTGAGTCTACN

File: PS19-800R.ab1 Run Ended: 2008/9/20 7:51:58 Signal G:803 A:618 C:1135 T:923
Sample: PS19_800R Lane: 22 Base spacing: 14.03 960 bases in 11315 scans



File: PS19-800R.ab1 *Run Ended: 2008/9/20 7:51:58* *Signal G:803 A:618 C:1135 T:923*

Sample: PS19_800R *Lane: 22* *Base spacing: 14.03* *960 bases in 11315 scans*



List of Publications

Research Articles

1. **Ahemad M**, Khan MS (2009) Toxicity assessment of herbicides quizalafop-p-ethyl and clodinafop towards *Rhizobium* pea symbiosis. **Bull Environ Contam Toxicol**, 82, 761-766

2. **Ahemad M**, Khan MS (2009) Phosphate-solubilizing and plant-growth-promoting *Pseudomonas aeruginosa* PS1 improves greengram performance in quizalafop-p-ethyl and clodinafop amended soil. **Arch Environ Contam Toxicol**, DOI 10.1007/s00244-009-9382-z

3. **Ahemad M**, Khan MS (2009) Effects of quizalafop-p-ethyl and clodinafop on plant growth promoting activities of rhizobacteria from mustard rhizosphere. **Ann Pl Protec Sci**, 17, 175-180

Review Article

Zaidi A, Khan MS, **Ahemad M**, Oves M (2009) Plant growth promotion by phosphate solubilizing bacteria. **Acta Microbiologica et Immunologica Hungarica**, 56, 263-284

Book Chapters

1. **Ahemad M**, Khan MS, Zaidi A, Wani PA (2009) Remediation Of Herbicides Contaminated Soil Using Microbes; In: Microbes in sustainable Agriculture, Khan MS, Zaidi A, Musarrat J (Editors), Nova Publishers, ISBN:978-1-60456-929-2, pp.261-284
2. Zaidi A, Khan MS, Wani PA, **Ahemad M** (2009) Bioremediation of heavy metals by plant growth promoting rhizobacteria; In: Microbes in sustainable Agriculture, Khan MS, Zaidi A, Musarrat J (Editors), Nova Publishers, ISBN:978-1-60456-929-2
3. Khan MS, Zaidi A, Wani PA, **Ahemad M**, Oves M (2009) Functional Diversity Among Plant Growth-Promoting Rhizobacteria: In: Microbial Strategies for Crop Improvement, Khan MS, Zaidi A, Musarrat J (Editors), Springer Berlin Heidelberg, pp. 105-132
4. Zaidi A, Khan MS, Wani PA, **Ahemad M**, Oves M (2009) Recent Advances in Plant Growth Promotion by Phosphate-Solubilizing Microbes: In: Microbial Strategies for Crop Improvement, Khan MS, Zaidi A, Musarrat J (Editors), Springer Berlin Heidelberg, pp. 23-50

5. **Ahemad M**, Zaidi A, Khan MS, Oves M (2009) Factors Affecting the Variation of Microbial Communities in Different Agro-Ecosystems: In: Microbial Strategies for Crop Improvement, Khan MS, Zaidi A, Musarrat J (Editors), Springer Berlin Heidelberg, pp. 301-324

Summary

Summary

In high input agronomic practices, pesticides are frequently used to prevent the organisms causing detrimental effect on crop plants and consequently to increase the crop productivity. After application, a large portion of pesticides persist in soils and pose a major threat to both microbial diversity and crop productivity. A well recognized practice for maintaining soil fertility has however, been the cultivation of legume crops, which add substantial amount of N to the soil by forming effective symbiosis with N_2 fixing organisms. Besides N_2 fixers, other plant growth promoting rhizobacteria (e.g. P solubilizers) are also used in agriculture production systems. And hence, microbial inoculants are commonly applied to seeds to ensure effective crop yields. The inoculant is, however, often used in conjunction with agrochemicals, which indeed contain essential nutrients to facilitate plant growth besides containing toxic substances. Of these chemicals, pesticides though applied to restrict the harmful effect of insect pests both in conventional and derelict soils may also be potentially hazardous and lead to losses in crop productivity.

Due to inadequate and conflicting reports on the toxicity of pesticides on plant growth promoting rhizobacteria and legume-*Rhizobium* symbiosis and the possibility of damage to the legumes due to the application of pesticides into the soils, it was desirable to explore the diversity of plant growth promoting rhizobacteria in terms of their functional variation in the Aligarh district of Western Uttar Pradesh, India. Subsequently, the toxicity of certain pesticides to the functional properties of selected PGPR and the effect of pesticides and pesticide tolerant PGPR strains on popularly grown legumes in the region was investigated. The present investigation was therefore, designed with specific objectives:-

1. To assess soil microbial diversity in different rhizospheres of popularly grown crops grown in this area.
2. To isolate nitrogen fixing bacteria from the nodules of legumes, chickpea, pea, greengram and lentil and phosphate solubilizing bacteria from mustard rhizosphere.
3. To evaluate the tolerance of rhizobacteria (nitrogen fixers and phosphate solubilizing bacteria) and growth pattern against selective herbicides (quizalafop-p-ethyl, clodinafop, metribuzin and glyphosate), insecticides (fipronil, pyriproxyfen, imidacloprid and thiamethoxam) and fungicides (tebuconazole, hexaconazole, metalaxyl and kitazin).

4. To assay both qualitatively and quantitatively the production of plant growth promoting substances by PGPR strains.

5. To assess the *in vitro* biotoxicity of pesticides to plant growth promoting traits of both nitrogen fixers and phosphate solubilizers and to identify pesticide tolerant strains for their PGP activities under pesticide stressed condition.

6. To evaluate the phytotoxic effects of recommended and higher doses of selective pesticides including herbicides (quizalafop-p-ethyl and clodinafop), insecticides (fipronil and pyriproxyfen) and a fungicide (tebuconazole) on the biological and chemical properties of chickpea, greengram, lentil and pea preferably grown in the vicinity of Aligarh.

7. To evaluate the effect of the pesticide tolerant plant growth promoting rhizobacteria on the performance of chickpea, greengram, lentil and pea plants grown in pesticide stressed soils.

The rhizospheric soils of chickpea, greengram, lentil, pea and mustard grown at the experimental fields of Faculty of Agricultural Sciences, A.M.U., Aligarh, were used to assess the microbial diversity. The bacterial populations in the rhizosphere of chickpea, greengram, lentil and pea were 3.21 x 10^7, 2.86 x 10^7, 3.53 x 10^7 and 3.11 x 10^7 CFU/g soil, respectively. The rhizospheric soils of mustard showed a substantial increase of 36, 52, 23 and 29% in bacterial populations compared to those recovered from chickpea, greengram, lentil, and pea, respectively. The fungal populations in all the rhizospheric soils ranged from 1.2 x 10^5 (lentil) to 2.1 x 10^5 (greengram) CFU/g soil. In general, the populations of phosphate solubilizing bacteria (PSB) were more than the phosphate solubilizing fungi (PSF) in all soil samples. Furthermore, a total of 50 N_2-fixing strains belonging to the genera *Mesorhizobium*, *Bradyrhizobium* and *Rhizobium* were isolated from the nodules of chickpea, greengram, lentil and pea crops using yeast extract mannitol agar plates while 50 strains of PSB were isolated from the rhizospheric soils of mustard. The isolated bacterial strains were characterized morphologically and biochemically. Among PSB, four isolates (PS1, PS2, PS9 and PS19) showing highest degree of TCP solubilization, were selected for further molecular characterization. These isolates were shown to belong to the genera *Pseudomonas aeruginosa* [PS1 (Gene Bank accession number FJ705886)], *Enterobacter asburiae* [PS2 (Gene Bank accession number FJ705887)], *Pseudomonas putida* [PS9 (Gene Bank accession number FJ705888) and *Klebsiella sp.* [PS19 (Gene Bank accession number FJ705889) by partial sequencing analysis of their respective 16s rDNA genes. Among the bacterial strains, 22% of *Mesorhizobium* spp. (chickpea), 18% of *Bradyrhizobium* spp.

(greengram), 14% of *Rhizobium* spp. (pea), 16% of *Rhizobium* spp. (lentil) and 36% of PSB were selected for assaying further the plant growth promoting activities. The mesorhizobial strains, rhizobial strains (pea), bradyrhizobial strains, rhizobial strains (lentil), phosphate solubilizing bacterial strains were grouped into four, three, three, four and four PGP groups, respectively.

The PGPR strains showing greatest plant growth promoting activities *in vitro* were selected to evaluate the toxic effects of varying concentrations of herbicides (quizalafop-p-ethyl, clodinafop, glyphosate, metribuzin), insecticides (fipronil, pyriproxyfen, imidacloprid, thiamethoxam), fungicides (tebuconazole, hexaconazole, metalaxyl and kitazin) using agar plate dilution method in order to identify the pesticide tolerant PGPR strains. Strains MRC4, MRP1, MRM6 and MRL3 showed the highest tolerance to most of the pesticides among *Mesorhizobium* spp., *Rhizobium* spp. (pea), *Bradyrhizobium* spp. and *Rhizobium* spp. (lentil), respectively. In contrast, of the 18 PSB, *Pseudomonas aeruginosa* PS1, *Enterobacter* asburiae PS2, *Pseudomonas putida* PS9 and *Klebsiella* sp. PS19, tolerated most of the tested pesticides. Of all the PGPR strains, *Pseudomonas aeruginosa* PS1 was the most tolerant bacterium which had a maximum resistance level (MRL) values for all the herbicides, insecticides and fungicides. Furthermore, growth pattern of the tolerant strains, *Mesorhizobium* strain MRC4, *Rhizobium* strain MRP1, *Bradyrhizobium* strain MRM6, *Rhizobium* isolate MRL3 and *Pseudomonas aeruginosa* PS1 grown in minimal media supplemented with different concentrations of 12 pesticides at different incubation periods showed a substantial variation.

The production of IAA by the selected bacterial strains namely, *Mesorhizobium* spp. (N=11), *Rhizobium* spp. (pea, N=7), *Bradyrhizobium* spp. (N=9), *Rhizobium* spp. (lentil, N=8) and PSB (N=18) was assayed in LB broth supplemented with a fixed concentration (100 µg/ml) of tryptophan. A wide range of variation in the secreted amount of IAA was observed among rhizobial isolates. Generally, the amount of IAA released by PGPR strains varied between 14 (MRC10) to 44 µg /ml (MRC4) for mesorhizobial strains, 17 (MRP4) to 32 µg /ml (MRP1) for pea specific *Rhizobium* isolates, 15 (MRM7) to 38 µg /ml (MRM6) for bradyrhizobial strains and 15 (MRL2, MRL7) to 37 µg/ml (MRL3) for *Rhizobium* strains isolated from lentil nodules. Of phosphate solubilizing bacteria (N=18), *Klebsiella* sp. PS19 was the most efficient strains and produced a highest amount of IAA (42 µg/ml) which was followed by *Pseudomonas aeruginosa* PS1 (39 µg/ml), *Pseudomonas putida* PS9 (34 µg/ml), *Enterobacter asburiae* PS2 (32 µg/ml) under normal growth conditions. Furthermore, a total of 36% of the *Mesorhizobium* strains

produced siderophore on CAS agar plates five days after incubation and the halo size for siderophores varied between 9 (MRC10) to 12 mm (MRC4). Further, the ethyl acetate extraction from culture supernatant of *Mesorhizobium* strain MRC1 yielded 30 and 17 µg/ml salicylate (SA) and 2,3-dihydroxy benzoic acid (DHBA), strain MRC4 produced 35 and 19 µg/ml of SA and DHBA, strain MRC7 yielded 25 and 18 µg/ml SA and DHBA, and strain MRC10 yielded 21 and 17 µg/ml SA and DHBA, respectively. Among the *Rhizobium* species isolated from pea nodules, only three (43%) strains were positive for siderophore activity where strain MRP1, MRP4 and MRP7 demonstrated 11, 10 and 11 mm orange colored zone on CAS agar plates. Further, these strains produced 32 and 22 (strain MRP1), 29 and 18 (MRP4) and 25 and 14 (MRP7) µg/ml SA and DHBA, respectively. Strains MRM3, MRM6 and MRM8 of *Bradyrhizobium* species showed 10, 13 and 11 mm colored zone, respectively, on CAS agar plates and produced 30 and 15 (strain MRM3), 32 and 18 (MRM6) and 28 and 16 (MRM8) µg/ml SA and DHBA, respectively. Among the *Rhizobium* species isolated from lentil nodules, 50% of the rhizobial isolates showed a positive reaction to siderophore both on CAS agar plates and in liquid culture medium. The siderophore zone size produced by such strains ranged between 10 (strain MRL1, MRL3, MRL7) to 12 mm (MRL6) and yielded 26 and 18 (MRL1), 29 and 21 (MRL3), 27 and 17 (MRL6) and 25 and 15 µg/ml SA and DHBA, respectively. Among the siderophore producing phosphate solubilizers (55%), *Pseudomonas aeruginosa* PS1, *Enterobacter asburiae* PS2, *Pseudomonas putida* PS9 and *Klebsiella* sp. PS19 had 15, 13, 14 and 14 mm colored zone, respectively on CAS plates. In liquid culture medium, *Pseudomonas aeruginosa* PS1 showed 41 and 21 µg/ml of SA and DHBA production. *Enterobacter asburiae* PS2 produced 24 and 9, *Pseudomonas putida* PS9 produced 41 and 17 and *Klebsiella* sp. PS19 produced 47 and 10 µg/ml of SA and DHBA, respectively.

The exo-polysaccharides (EPS) synthesized by the pesticide tolerant bacterial strains were determined after 120 h of incubation. Among the bacterial strains, a total of 36, 43, 33, 38 and 100% of mesorhizobia, rhizobia (pea), bradyrhizobia, rhizobia (lentil) and PSB respectively, secreted EPS in liquid culture medium. Particularly, *Mesorhizobium* strain MRC4, *Rhizobium* strain MRP1, *Bradyrhizobium* strain MRM6, *Rhizobium* strain MRL3, *Pseudomonas aeruginosa* PS1, *Enterobacter asburiae* PS2, *Pseudomonas putida* PS9 and *Klebsiella* sp. PS19 produced 21, 20, 21, 18, 18, 16, 17 and 18 µg/ml EPS, respectively. The plant growth promoting rhizobacteria were further evaluated for their phosphate solubilizing potential, both on solid and in liquid

Pikovskaya medium. A total of 34% rhizobacterial strains showed the phosphate solubilizing activity and formed a clear halo around their growth. Generally, the size of phosphate solubilizing zone on solid Pikovskaya ranged from 4 (*Bacillus* sp. PS4) to 14 mm (*Klebsiella* sp. PS19). The solubilization index (SI) ranged between 0.5 (*Bacillus* PS 4) to 2.5 (*Klebsiella* PS 19). Similarly, a considerable amount of tri-calcium phosphate (TCP) was solubilized in liquid culture by *Pseudomonas aeruginosa* PS1 (345 µg/ml), *Enterobacter asburiae* PS2 (258 µg/ml), *Pseudomonas putida* PS9 (298 µg/ml) and *Klebsiella* sp. PS19 (294 µg/ml). The solubilization of TCP was accompanied by decrease in pH of the medium. In addition, all growth promoting rhizobacterial strains of *Bradyrhizobium*, *Rhizobium* (lentil) and phosphate solubilizers showed a positive reaction for ammonia. In contrast, only 91% of *Mesorhizobium* and 86% of *Rhizobium* (pea) were positive for ammonia Furthermore, a total of 63% *Mesorhizobium*, 100% *Rhizobium* (pea), 33% *Bradyrhizobium*, 75% *Rhizobium* (lentil) and 50% phosphate solubilizing strains were found to be positive for HCN production.

A total of eight pesticide tolerant rhizobacterial strains including *Mesorhizobium* strain MRC4, *Rhizobium* strain MRP1 (pea), *Bradyrhizobium* strain MRM6, *Rhizobium* strain MRL3 (lentil), *Pseudomonas aeruginosa* strain PS1, *Enterobacter asburiae* strain PS2, *Pseudomonas putida* strain PS9 and *Klebsiella sp.* strain PS19 were evaluated further for plant growth promoting activities in their respective medium supplemented with varying concentrations of selected pesticides. In this study, the effect of three concentrations (recommended dose – X, double of recommended dose – 2X and three times more of recommended dose – 3X) of herbicides (quizalafop-p-ethyl, clodinafop, metribuzin and glyphosate), insecticides (fipronil, pyriproxyfen, imidacloprid and thiamethoxam) and fungicides (tebuconazole, hexaconazole, metalaxyl and kitazin) on IAA synthesized by rhizobacterial strains was determined in LB broth treated with 100 µg/ml of tryptophan. The rhizobacterial strains in general through produced a considerable amount of IAA but IAA decreased progressively with increase in concentrations of herbicides, insecticides and fungicides. In case of herbicides, metribuzin had the least toxic effect on IAA synthesis while quizalafop-p-ethyl had a profound adverse effect on IAA production by mesorhizobial strain MRC4. Of all the herbicides, metribuzin reduced the IAA production by 16% while quizalafop-p-ethyl by 75% at 3X concentration, over untreated control. Among insecticides, pyriproxyfen affected IAA synthesis most severely and decreased it by 62% while fungicide tebuconazole decreased it by 75% at 3X, relative to control. Among herbicides,

quizalafop-p-ethyl displayed most toxic effect on IAA produced by *Rhizobium* strain MRP1 and decreased it by 44% at 3X over control. Among insecticides, fipronil showed highest toxicity and decreased IAA production by 35% at 3X in comparison to control while tebuconazole, among fungicides, demonstrated the greatest toxicity on IAA and declined it by 50% at 3X, compared to control. *Bradyrhizobium* strain MRM6 when grown in LB medium amended with normal rates of quizalafop-p-ethyl, clodinafop, metribuzin and glyphosate produced maximum amount of 7, 17, 30 and 28 µg/ml IAA which significantly declined by 8%, 18%, 32% and 40% respectively, at 3X of each herbicide over control. Among three concentrations of each herbicide, the 3X of quizalafop-p-ethyl was most toxic and reduced the production of IAA by 57% compared to those observed for normal rate of the same herbicide. Of the three concentrations of each insecticide, the 3X of both fipronil and pyriproxyfen showed the most toxic effect and reduced the IAA biosynthesis by 56% relative to those observed for recommended rates of the same insecticides. Like the effect of 3X of both herbicides and insecticides, the 3X of fungicides in general, also had a greatest toxic effect on IAA synthesis by *Bradyrhizobium* MRM6; the maximum being observed for 3X of tebuconazole which reduced IAA by 89% over untreated control. In the presence of different concentrations of herbicides insecticides and fungicides, IAA production by *Rhizobium* strain MRL3 decreased progressively as the concentration of each pesticide was increased gradually from recommended to the highest tested dose.

Phosphate solubilizing bacteria namely, *Pseudomonas aeruginosa* PS1, *Enterobacter asburiae* PS2, *Pseudomonas putida* PS9 and *Klebsiella* sp. PS19 tolerant to herbicides, insecticides and fungicides were also tested for IAA synthesis under pesticide stressed environment. Even though, these bacterial strains produced IAA but in general, the synthesis of IAA by the P-solubilizers decreased consistently with increasing concentrations of herbicides, insecticides and fungicides. However, production of IAA by the four selected pesticide tolerant and P solubilizing strains (*Pseudomonas aeruginosa* PS1, *Enterobacter asburiae* PS2, *Pseudomonas putida* PS9 and *Klebsiella* sp. PS19) under pesticides stress conditions did not differ significantly. The biotoxicity of quizalafop-p-ethyl among herbicides, pyriproxyfen within insecticides and tebuconazole in fungicides group was most prominent over bacterial IAA biosynthesis. Quizalafop-p-ethyl decreased the synthesis of IAA by 90, 91, 88 and 84%, pyriproxyfen by 85, 72, 80 and 79% and tebuconazole by 92, 94, 95 and 93% at 3X by *Pseudomonas aeruginosa* PS1, *Enterobacter asburiae* PS2, *Pseudomonas putida* PS9 and

Klebsiella sp. PS19, respectively. Though the order of biotoxicity of pesticides on bacterial IAA biosynthesis was not uniform, however, at three times of recommended dose, the order of toxicity of pesticides on the IAA synthesis by *Pseudomonas aeruginosa* PS1, the most tolerant PSB strain to all herbicides, insecticides and fungicides was: quizalafop-p-ethyl > clodinafop > glyphosate > metribuzin; pyriproxyfen > imidacloprid > fipronil > thiamethoxam and tebuconazole > hexaconazole > metalaxyl > kitazin, respectively.

Production of siderophores by the pesticide tolerant strains of PGPR was also determined both qualitatively and quantitatively in the medium supplemented with or without varying concentrations of pesticides. Generally, the tested PGPR strains showed siderophore activity on pesticides amended CAS agar plates. The size of siderophore zone produced on CAS agar plates by PGPR strains decreased with increasing concentrations of each pesticide. Furthermore, the amount of SA and DHBA, respectively, in the supernatant of *Mesorhizobium* strain MRC4 decreased consistently with increasing dose of each pesticide. Quizalafop-p-ethyl at 3X have shown maximum toxicity and decreased SA and DHBA by 46% and 48% respectively, compared to control. Among insecticides, the most prominent inhibitory effect on SA and DHBA production was recorded for pyriproxyfen which decreased SA and DHBA by 40% and 37% at 3X. Among fungicides, tebuconazole and hexaconazole affected the siderophores activity most severely. Tebuconazole reduced the production of SA and DHBA by 40 and 58% while hexaconazole by 40 and 48% respectively, at 3X over control. Additionally, the amount of SA and DHBA in the supernatant of *Rhizobium* strain MRP1 decreased consistently with increasing dosage of each pesticide. Of all the herbicides, quizalafop-p-ethyl displayed maximum toxicity and decreased SA by 57% while it reduced the DHBA by 55% at 3X, over control. The most toxic effect on SA and DHBA production was shown, among insecticides, by pyriproxyfen which decreased SA by 35% and DHBA by 46% at 3X,. Fipronil and thiamethoxam slightly reduced the siderophore activity and showed a similar pattern of SA and DHBA inhibition following strain MRP1 inoculation. Among fungicides, tebuconazole affected the siderophores production most severely and inhibited SA by 44% and DHBA by 60% at 3X, relative to control. Besides, quizalafop-p-ethyl at 3X displayed the maximum toxicity and decreased SA by 62 and 48% and DHBA by 72 and 57% for *Bradyrhizobium* strain MRM6 and *Rhizobium* strain MRL3, respectively, over respective control. Likewise, pyriproxyfen at 3X showed maximum toxicity and decreased SA by 34 and 28% and DHBA by 33 and 57% for

Bradyrhizobium strain MRM6 and *Rhizobium* strain MRL3, respectively, compared to their respective control. On the other hand, tebuconazole at 3X decreased both SA and DHBA by 44 and 52% for *Bradyrhizobium* strain MRM6 and *Rhizobium* strain MRL3, over their control.

Phosphate solubilizing bacterial strains *Pseudomonas aeruginosa* PS1, *Enterobacter asburiae* PS2, *Pseudomonas putida* PS9 and *Klebsiella* sp. PS19 tolerant to pesticides were also tested for siderophore production which decreased consistently with increasing concentrations of pesticides. Among all herbicides, quizalafop-p-ethyl at 3X displayed the maximum decrease in production of SA by 35, 68, 46 and 47% and of DHBA by 48, 78, 89 and 90% for *Pseudomonas aeruginosa* PS1, *Enterobacter asburiae* PS2, *Pseudomonas putida* PS9 and *Klebsiella* sp. PS19, respectively, over respective control. Similarly, among insecticides, pyriproxyfen at 3X showed maximum biotoxicity to SA and decreased it by 52, 47, 36 and 47% and to DHBA by 80, 83, 67 and 70% for *Pseudomonas aeruginosa* PS1, *Enterobacter asburiae* PS2, *Pseudomonas putida* PS9 and *Klebsiella* sp. PS19, respectively, compared to their respective control. Tebuconazole (fungicide) at 3X, decreased SA to highest degree by 54, 69, 58 and 52% and DHBA by 70, 77, 67 and 77% for *Pseudomonas aeruginosa* PS1, *Enterobacter asburiae* PS2, *Pseudomonas putida* PS9 and *Klebsiella* sp. PS19, respectively over control. Exo-polysaccharides (EPS) synthesized by all PGPR strains increased progressively with gradual enhancement in pesticide concentrations. For instance, glyphosate at 3X, among herbicides, increased the EPS by 23% over control. Of insecticides, imidacloprid increased EPS by 38% while fungicide hexaconazole by 33% at 3X compared to control. For *Rhizobium* strain MRP1 specific to pea, glyphosate, pyriproxyfen and tebuconazole increased EPS by 40, 30 and 25% respectively, at 3X over control. On the other hand, for *Bradyrhizobium* strain MRM6, glyphosate increased EPS by 38%, fipronil, pyriproxyfen and thiamethoxam by 23%, tebuconazole and hexaconazole by 28% at 3X compared to control. Unlike the marginal increment in EPS synthesis by *Rhizobium* strain MRL3 (lentil) in the presence of metribuzin and glyphosate, both quizalafop-p-ethyl and glyphosate at 3X increased EPS by 33% when compared with untreated control. Similarly, imidacloprid at 3X substantially increased EPS secretion by 44% compared to control. On the other hand, hexaconazole at three times of recommended rate, was found the most potential inducer of bacterial EPS secretion and increased it by 50% over control. The synthesis of EPS by the P solubilizers increased consistently with increasing concentration of each herbicides, insecticides and fungicides. The trend of EPS production by P solubilizing strains like

Pseudomonas aeruginosa PS1, *Enterobacter asburiae* PS2, *Pseudomonas putida* PS9 and *Klebsiella* sp. PS19 under pesticide stress was not uniform. In general, the effect of glyphosate (among herbicides), pyriproxyfen (insecticides) and hexaconazole (among fungicides) on bacterial EPS secretion was most obvious compared to their respective control. For example, glyphosate increased the synthesis of EPS by 38, 43, 47 and 38%, pyriproxyfen by 50, 37, 35 and 33% and hexaconazole by 56, 55, 41 and 61% at 3X by *Pseudomonas aeruginosa* PS1, *Enterobacter asburiae* PS2, *Pseudomonas putida* PS9 and *Klebsiella* sp. PS19, respectively over their respective control.

The rhizobacterial strains were further tested for HCN and ammonia production under *in vitro* conditions in the presence of three concentrations of twelve pesticides. Interestingly, the three concentrations of herbicides, insecticides and fungicides did not affect negatively HCN and ammonia synthesis each by *Mesorhizobium*, *Rhizobium* specific to pea, *Bradyrhizobium*, *Rhizobium* specific to lentil and phosphate solubilizing strains of *Pseudomonas aeruginosa* PS1, *Enterobacter asburiae* PS2, *Pseudomonas putida* PS9 and *Klebsiella* sp. PS19. The phosphate solubilizing potentials of the PGPR strains in the presence of varying concentrations of herbicides, insecticides and fungicides was also assayed both qualitatively and quantitatively using Pikovskaya medium. In this study, the phosphate solubilizing bacteria, *Pseudomonas aeruginosa* (strain PS1), *Enterobacter asburiae* (strain PS2), *Pseudomonas putida* (strain PS9) and *Klebsiella* sp. (strain PS19) were used due to their inherent ability to tolerate the highest concentration of pesticides and production of PGP substances in maximum amounts. All these strains produced a largest zone of P solubilization around their growth on solid Pikovskaya medium devoid of pesticides whose solubilization index (SI) ranged between 2 (*Pseudomonas aeruginosa* PS1) and 2.5 (*Klebsiella* sp. PS19). In contrast, the zone of solubilization and *in vitro* solubilization of tri-calcium phosphate (TCP) decreased substantially when PGPR strains were grown with 3X concentrations each of herbicides, insecticides and fungicides. For example, quizalafop-p-ethyl decreased the solubilization zone by 25, 73, 58 and 45%, pyriproxyfen by 38, 50, 43 and 40% and tebuconazole by 25, 63, 58 and 60% at 3X for *Pseudomonas aeruginosa* PS1, *Enterobacter asburiae* PS2, *Pseudomonas putida* PS9 and *Klebsiella* sp. PS19 respectively, over their control. Similar to the effects of pesticides on solubilizing zones, a maximum reduction in P solubilization in broth was found as 96, 82 and 96% by *Pseudomonas aeruginosa* PS1, 95, 87 and 94% by *Enterobacter asburiae* PS2, 95, 93 and 95% by *Pseudomonas putida*

PS9 and 97, 96 and 95% by *Klebsiella* sp. PS19 at 3X of quizalafop-p-ethyl, pyriproxyfen and tebuconazole respectively, over their respective control.

Soils contaminated with pesticides present a major concern for sustainable agriculture. In addition, legumes are used as a rich source of protein in Indian dietary systems, and hence, understanding the effects of pesticides on the legume productivity will be useful. Therefore, the phytotoxic effects of the recommended (X), two (2X) and three (3X) times more of recommended rates of technical grade herbicides (quizalafop-p-ethyl and clodinafop), insecticides (fipronil and pyriproxyfen) and fungicide (tebuconazole) on the biological and chemical characteristics of chickpea, pea, lentil and greengram in pot trials was studied. The rhizobial strains *Mesorhizobium* MRC4, *Rhizobium* MRP1, *Bradyrhizobium* MRM6, *Rhizobium* MRL3 and phosphate solubilizing bacterium *Pseudomonas aeruginosa* PS1 resistant to herbicides (quizalafop-p-ethyl, clodinafop, metribuzin, and glyphosate), insecticides (fipronil, pyriproxyfen, imidacloprid, and thiamethoxam) and fungicides (tebuconazole, hexaconazole, metalaxyl and kitazin) and producing the plant growth promoting substances substantially even in pesticide stress, were used to determine their bioremediation potential using chickpea, pea, lentil and greengram as test crop, when grown in the soil treated with or without herbicides, insecticides and fungicides.

The length of plant organs (roots and shoots) of chickpea grown in sandy clay loam soil treated with the recommended, two and three times more of recommended rates of technical grade herbicides (quizalafop-p-ethyl and clodinafop), insecticides (fipronil and pyriproxyfen) and fungicide (tebuconazole) was measured at 90 and 135 days after sowing (DAS). Generally, a progressive decline with variable magnitude was observed for both roots and shoots length as the concentration of all pesticides was increased from X to 3X in soil. For example, quizalafop-p-ethyl at 3X (120 µg/ kg soil) displayed the most toxic effect and decreased roots length and shoots length by 72 and 53%, respectively (at 90 DAS) and by 73 and 55%, respectively (at 135 DAS) over control. A considerable enhancement was observed in roots and shoots length of inoculated chickpea plants when compared with the uninoculated plants grown in soils treated with the similar concentration of pesticides. For example, when strain MRC4 of *Mesorhizobium* was used with 3X of quizalafop-p-ethyl, it increased the root and shoot length by 42 and 12%, respectively (at 90 DAS) and 22 and 17%, respectively (at 135 DAS) compared with the uninoculated plants grown in soil treated with the same dose of quizalafop-p-ethyl. The

phytotoxicity of pesticides to dry biomass production by plant organs (roots and shoots) and total dry matter accumulation in chickpea plants consistently decreased with increasing concentrations of herbicides, insecticides and fungicides when applied separately. In general, three concentrations each of X, 2X and 3X of all pesticides significantly ($P \leq .05$) decreased the dry matter accumulation both at 90 DAS and 135 DAS, relative to the control. For example, at recommended dose, tebuconazole (100 µg/ kg soil) reduced the root and shoot dry biomass by 48 and 63%, respectively (at 90 DAS) and by 29 and 53%, respectively (at 135 DAS) over control. However, *Mesorhizobium* strain MRC4 increased the shoot dry matter by 30, 28 and 15% at 90 DAS and 82, 16, 14% at 135 DAS at three times of the recommended rates of quizalafop-p-ethyl, fipronil and tebuconazole respectively, compared to uninoculated plants grown in soil treated with the same dose of quizalafop-p-ethyl, fipronil and tebuconazole.

Nodulation response to the three concentrations of quizalafop-p-ethyl, clodinafop, fipronil, pyriproxyfen and tebuconazole at 90 and 135 DAS varied considerably. Generally, each pesticide decreased the nodule numbers and its biomass when concentration of pesticide was increased from normal to three times more of recommended rates. Like plant dry matter accumulation, quizalafop-p-ethyl among herbicides, showed highest toxicity at three times of recommended dose (120 µg/ kg soil) and decreased nodule numbers by 67 and 65% and nodule biomass by 87 and 79% at 90 and 135 DAS, respectively, compared to control. Similarly, pyriproxyfen (insecticide) at 1300 µg/ kg soil (X), 2600 µg/ kg soil (2X) and 3900 µg/ kg soil (3X) adversely affected the chickpea-*Mesorhizobium* symbiosis and decreased nodule numbers by 5, 10 and 14% and nodule mass by 24, 34 and 42% respectively, above the control at 90 DAS. Tebuconazole, at X (100 µg/ kg soil), 2X (200 µg/ kg soil) and 3X (300 µg/ kg soil, also severely affected nodulation and reduced nodule numbers by 24, 34 and 48% and nodule mass by 36, 47 and 56% respectively, at 90 DAS while 3X of the same fungicide declined nodule numbers and nodule dry biomass by 36% and 73% respectively, at 135 DAS compared to control. It was interesting to observe that bioinoculant, in general, significantly ($P \leq 0.05$) improved the nodulation on chickpea plants when grown even in the presence of each class of pesticides. As an example, when strain MRC4 was used with pyriproxyfen at 1300 µg/ kg soil (X), 2600 µg/ kg soil (2X) and 3900 µg/ kg soil (3X), it increased the nodule numbers by 14, 31 and 16% and nodule dry mass by 25, 34 and 42% at 90 DAS while at 135 DAS, it enhanced nodule numbers by 162, 233 and 183% and nodule biomass by 52, 64 and 75%, respectively. The

leghaemoglobin and total chlorophyll content consistently declined with increasing rates of pesticides either in the presence or absence of inoculant and was significant for all pesticides. At highest concentration (3X) of herbicides added to soil, 120 µg/ kg soil of quizalafop-p-ethyl and 1200 µg/ kg soil of clodinafop decreased leghaemoglobin equally by 93% and chlorophyll by 34% and 13% respectively, above control. In addition, pyriproxyfen at 3X (3900 µg/ kg soil) decreased leghaemoglobin and chlorophyll content most severely by 77 and 16%, respectively, which was followed by fipronil that reduced leghaemoglobin and chlorophyll content by 85 and 21%, respectively, over control. In contrast, tebuconazole decreased leghaemoglobin and chlorophyll content by 93 and 28%, respectively, over control. Interestingly, strain MRC4 with 3X of quizalafop-p-ethyl (120 µg/ kg soil), clodinafop (1200 µg/ kg soil), fipronil (600 µg/ kg soil), pyriproxyfen (3900 µg/ kg soil) and tebuconazole (300 µg/ kg soil) increased leghaemoglobin and chlorophyll content by 0% and 45%, 130% and 26%, 133% and 32%, 150% and 32% and 100% and 37%, respectively, compared to uninoculated plants treated with the same dose of quizalafop-p-ethyl, clodinafop, fipronil, pyriproxyfen and tebuconazole, respectively.

Nitrogen (N) and phosphorus (P) content in roots and shoots, seed yield (SY) and grain protein (GP) of chickpea plants was measured at harvest (135 DAS). The measured parameters decreased progressively with increase in the concentration of each pesticide. For instance, at three times of recommended dose, the percent decrease in root N, shoot N, root P, shoot P, SY and GP in presence of clodinafop (at 1200 µg/ kg soil) was 23, 15, 36, 29, 38 and 7, respectively, compared to the control. In contrast, the inoculated strain (MRC4) significantly increased the root N, shoot N, root P, shoot P, SY and GP at all concentration of pesticides relative to uninoculated plants treated with the same dose of pesticides.

Pea plants grown in sandy clay loam soil treated with three concentrations each of quizalafop-p-ethyl, clodinafop, fipronil, pyriproxyfen and tebuconazole 90 and 120 DAS showed a variable plant growth. A pattern of progressive decline with variable degree was recorded for both roots and shoots length as the concentrations of three classes of pesticides were increased in soils. The decreasing order of phytotoxicity of pesticides on the length of plant organs was: quizalafop-p-ethyl > tebuconazole > pyriproxyfen > fipronil > clodinafop. A substantial improvement was observed in roots and shoots length of pea plants inoculated with strain MRP1 when compared with the uninoculated treatments having the same concentration of pesticides. The dry biomass

of roots and shoots continuously decreased as the concentration of each pesticide was increased. Among herbicides, quizalafop-p-ethyl at recommended dose decreased roots and shoots dry mass by 49 and 52%, respectively. Among insecticides, pyriproxyfen decreased roots and shoots dry mass by 29 and 37%, respectively, while fipronil mediated decline in roots and shoots dry biomass was 18 and 34% respectively, at normal rate at 90 DAS over control. Moreover, reduction in roots and shoots dry biomass by fungicide tebuconazole at X was 38 and 54%, respectively at 90 DAS, relative to control. However, in the presence of bioinoculant, the severity of pesticide generated toxicity on biomass accumulation was substantially decreased. For instance, strain MRP1 when used with 3X of tebuconazole, increased the roots dry matter by 81% and shoots dry mass by 60% at 90 DAS. Substantial variation was observed in nodule numbers and nodule dry mass of pea plants grown in soils amended with three concentrations each of quizalafop-p-ethyl, clodinafop, fipronil, pyriproxyfen and tebuconazole at both 90 and 120 DAS. With increase in the concentration of each pesticide, there was a substantial decrease in nodulation. In case of some pesticides, nodule formation was completely diminished when pea plants were grown in soils treated with higher concentration of pesticides. For example, quizalafop-p-ethyl at recommended rate decreased nodule numbers and nodule dry weight by 75 and 48%, respectively, over the control at 90 DAS while at 120 DAS it completely abolished nodulation. Moreover, bioinoculant MRP1 significantly improved the nodulation on pea plants when grown even in the presence of pesticides. For instance, strain MRP1 with tebuconazole at 2X increased nodule numbers by 11% and nodule dry mass by 47% at 90 DAS compared to uninoculated plants grown in soils treated with same dose of tebuconazole. Leghaemoglobin content in fresh nodules and total chlorophyll content in foliage measured at 90 DAS, progressively decreased with increasing concentration of each pesticide both in the presence or the absence of bioinoculant. Quizalafop-p-ethyl, clodinafop, fipronil, pyriproxyfen and tebuconazole at 3X decreased leghaemoglobin and chlorophyll content by 100 and 16%, 24 and 8%, 36 and 10%, 36 and 15% and 100 and 14% respectively, over control. However, a substantial increase in leghaemoglobin and chlorophyll content was observed when inoculated plants were compared with the uninoculated ones treated with the same concentration of pesticides. For illustration, *Rhizobium* strain MRP1, when used with fipronil (at three times more of recommended dose), increased leghaemoglobin and chlorophyll content by 36 and 19% respectively, compared to uninoculated plants treated with the same dose of fipronil. Nitrogen

and phosphorus content, seed yield and grain protein of pea plants measured at harvest, decreased gradually with increasing concentrations of each pesticide. At three times of recommended dose, the percent decrease in root N, shoot N, root P, shoot P, SY and GP was 24, 36, 39, 36, 50 and 4 for quizalafop-p-ethyl; 15, 29, 24, 18, 14 and 2 for clodinafop; 27, 27, 29, 18, 15 and 2 for fipronil; 27, 20, 29, 25, 15 and 2 for pyriproxyfen and 26, 32, 34, 33, 23 and 3 for tebuconazole, respectively, compared to the control. In contrast, when strain MRP1 was used with pesticides, severity of toxicity of all pesticides on these parameters was less pronounced.

Uninoculated and *Bradyrhizobium* inoculated greengram plants grown in soil treated separately with three concentrations each of quizalafop-p-ethyl, clodinafop, fipronil, pyriproxyfen and tebuconazole showed considerable variation in pot house experiments. The length of roots and shoots of greengram plants declined consistently following increase in concentration of pesticides. However, no significant differences were observed for the measured parameters while comparing the effect of pesticides at 50 or 80 days old greengram plants. For instance, clodinafop at 3X, decreased the root length by 65% at 50 DAS while at 80 DAS, it decreased root length by 64%. Similarly, 3X of clodinafop decreased shoots length by 50% at 50 DAS and 63% at 80 DAS relative to control. For plant growth promoting and pesticide tolerant *Bradyrhizobium* (strain MRM6) and *Pseudomonas aeruginosa* (strain PS1) inoculated plants, the roots and shoots length also decreased continuously with increasing concentration of pesticides but significant enhancement was found in the measured parameters of greengram plants when compared with the uninoculated plants grown in soils treated with the same concentration of pesticides. The dry matter accumulation in roots, shoots and the whole greengram plants were adversely affected in response to pesticide application. Generally, all concentrations of pesticides significantly ($P \leq 0.05$) decreased the dry matter accumulation of whole greengram plants both at 50 and 80 DAS, relative to control. For example, tebuconazole at recommended rate decreased roots and shoots dry mass by 16% and 26% respectively, at 50 DAS. The pesticidal toxicity onto greengram plants increased in the order: quizalafop-p-ethyl > tebuconazole > pyriproxyfen > fipronil > clodinafop. Moreover, roots and shoots dry mass at each dose rate of all pesticides increased appreciably when inoculated plants were compared to the uninoculated ones. For example, pesticide tolerant *Bradyrhizobium* strain MRM6 when applied with X, 2X and 3X of clodinafop, significantly ($P \leq 0.05$) increased the root dry matters by 47, 60 and 68% respectively, and shoot dry weight by 48, 55 and 61% respectively, at 50 DAS. While at 80 DAS,

it enhanced the dry matter accumulation in roots by 50, 58 and 62% respectively and shoots dry weight by 7, 13 and 24% at X, 2X and 3X of clodinafop, compared to uninoculated greengram plants grown in soils treated with the same concentration of clodinafop. Furthermore, *Pseudomonas aeruginosa* strain PS1 with 3X of pyriproxyfen dramatically increased the roots dry matter by 247% and shoots dry weight by 413% at 50 DAS while at 80 DAS, it increased roots dry biomass by 447% and shoot dry weight by 513% compared to uninoculated greengram plants treated with the same concentration of pyriproxyfen.

With increasing concentrations of each pesticide, nodule numbers and their dry weight decreased progressively in both presence and absence of bioinoculant. For uninoculated plants, quizalafop-p-ethyl, clodinafop, fipronil, pyriproxyfen and tebuconazole at recommended dose decreased nodule numbers by 29, 10, 10, 15 and 17%, while nodule dry weight by 38, 8, 13, 24 and 25% respectively, at 50 DAS relative to control. In general, quizalafop-p-ethyl displayed the most lethal effect on nodulation. Moreover, bioinoculant strain MRM6 increased the nodule numbers and their mass extensively at all concentrations of each pesticide. For instance, pyriproxyfen at 3X increased nodule numbers and nodule dry mass by 33 and 172% respectively, at 50 DAS while at 80 DAS by 62 and 153% respectively, when compared to the uninoculated plants grown in soils treated with 3X of pyriproxyfen. Also, *Pseudomonas aeruginosa* strain PS1 with clodinafop at 3X increased nodule numbers by 156 and nodule dry mass by 178% at 50 DAS while at 80 DAS, nodule numbers by 63 and nodule dry mass by 293% compared to the uninoculated greengram plants treated with the same dose of clodinafop. Leghaemoglobin and chlorophyll content measured at 50 DAS consistently declined with increasing concentration of each pesticide both in the presence or the absence of the inoculant. For instance, quizalafop-p-ethyl, clodinafop, fipronil, pyriproxyfen and tebuconazole at 3X decreased leghaemoglobin and chlorophyll content by 63 and 25%, 38 and 9%, 38 and 13%, 50 and 14% and 50 and 15% respectively, relative to control. Nevertheless, when *Bradyrhizobium* strain MRM6 when applied with clodinafop at two times more of recommended dose, increased leghaemoglobin and chlorophyll content by 33 and 14% respectively, compared to uninoculated plants at the same dose of clodinafop. Similarly, *P. aeruginosa* strain PS1 with recommended dose of tebuconazole increased leghaemoglobin and chlorophyll content by 14 and 12% respectively, compared to the uninoculated plants raised in soils treated with same dose of tebuconazole. Nitrogen and phosphorus content, seed yield and grain protein measured at 80 DAS decreased regularly with

increasing dose rate of each pesticide both in the presence and the absence of inoculant. For example, at three times of recommended rate, the percent decrease in root N, shoot N, root P, shoot P, SY and GP in the presence of quizalafop-p-ethyl was 45, 44, 52, 37, 63 and 12; 17, 16, 15, 20, 29 and 4 for clodinafop; 34, 22, 23, 14, 38 and 5 for fipronil; 37, 32, 38, 25, 40 and 7 for pyriproxyfen and 25, 30, 38, 34, 49 and 8 for tebuconazole, respectively, compared to control. Interestingly, the inoculant strains (*Bradyrhizobium* strain MRM6 and *P. aeruginosa* strain PS1) significantly ($P \leq 0.05$) increased the root N, shoot N, root P, shoot P, SY and GP at all concentration of pesticides. For instance, *Bradyrhizobium* strain MRM6 when used with 3X of fipronil, increased the root N, shoot N, root P, shoot P, SY and GP by 29, 31, 10, 0, 78 and 5% respectively, compared to the treatment with 3X of fipronil but lacking inoculant. Similarly, *P. aeruginosa* strain PS1 when used with 3X of clodinafop, increased the root N, shoot N, root P, shoot P, SY and SP by 27, 38, 13, 34, 83 and 3%, respectively, when compared to the treatment having the same dose of clodinafop but without inoculant.

All pesticides showed the phytotoxicity and reduced of the length of both roots and shoots of lentil plants progressively, as the concentration of pesticides was increased in soils from recommended to three times more of recommended rate. In general, quizalafop-p-ethyl affected adversely the growth of roots and shoots of lentil plants. Moreover, the effects of other pesticides like fipronil, pyriproxyfen and tebuconazole on root and shoot length was comparable. Substantial increase in root and shoot length of *Rhizobium* strain MRL3 inoculated lentil plants occurred compared to the uninoculated plants treated with the same concentration of pesticides. For example, strain MRL3 in the presence of tebuconazole (at three times of recommended dose), increased the root and shoot length by 43 and 27% respectively, at 90 DAS while at 120 DAS by 8% and 17% respectively, compared to the uninoculated plants treated with the same dose of tebuconazole. At recommended dose, quizalafop-p-ethyl, clodinafop, fipronil, pyriproxyfen and tebuconazole decreased roots and shoots dry biomass by 40 and 45, 10 and 8, 19 and 19, 32 and 24 and 28 and 36% respectively, at 90 DAS while at 120 DAS by 52 and 41, 7 and 6, 22 and 11, 26 and 16 and 32 and 24% respectively, over control. Maximum decline in plant root (68 and 71%) and shoot (65 and 63%) dry matters was shown by quizalafop-p-ethyl (3X) at 90 and 120 DAS, respectively. Effect of other pesticides was however, comparatively less inhibitory. Furthermore, *Rhizobium* strain MRL3 significantly increased the dry biomass at all dose rates of pesticides when inoculated lentil plants were compared to the uninoculated

plants. For example, strain MRL3 with X, 2X and 3X of clodinafop increased the shoots dry matter by 67, 80 and 60% respectively, at 90 DAS while at 120 DAS by 76, 75 and 80%, compared to uninoculated treatments treated with the same dose rates of clodinafop.

Nodulation in lentil plants grown in soils treated with the three concentrations each of quizalafop-p-ethyl, clodinafop, fipronil, pyriproxyfen and tebuconazole at 90 and 120 DAS varied considerably. Like other legume crops tested in this study, the nodule numbers and the dry weight of lentil nodules also declined when concentration of pesticide was increased from X to 3X. A least toxic effect on nodulation was however, shown by clodinafop which at X, 2X and 3X, decreased the nodule numbers by 6, 11 and 37% (at 90 DAS) and by 3, 19 and 27% (at 120 DAS) and nodule dry mass by 7, 20 and 30% (at 90 DAS) and 7, 17 and 32% (at 120 DAS), respectively. Quizalafop-p-ethyl at 3X showed maximum toxicity among pesticides and decreased both nodule numbers and nodule biomass equally by 100 and 64% at both 90 and 120 DAS, respectively. However, the bioinoculant significantly increased the nodule numbers and nodule biomass when compared to uninoculated treatments of the same concentration. For example, *Rhizobium* strain MRL3 with fipronil at X, 2X and 3X, increased nodule numbers by 50, 38 and 50% respectively, and nodule dry mass by 108, 86 and 84% respectively, at 90 DAS while at 120 DAS, nodule numbers by 3, 7 and 11% respectively, and nodule biomass by 23, 36 and 60%, respectively, compared to the uninoculated plants treated with the same dose of fipronil. The leghaemoglobin and total chlorophyll content also consistently decreased with increasing concentrations of pesticides. At recommended dose, quizalafop-p-ethyl, clodinafop, fipronil, pyriproxyfen and tebuconazole decreased leghaemoglobin and chlorophyll content by 100 and 22%, 9 and 4%, 17 and 7%, 25 and 4% and 34 and 13% respectively, relative to control. However, substantial increase in leghaemoglobin and chlorophyll content was observed when inoculated plants were compared with the uninoculated ones grown in soils amended with same concentration of pesticides. Nitrogen and phosphorus content, seed yield and grain protein (GP) of lentil plants measured at harvest (120 DAS) decreased progressively with increasing concentration of each pesticide from X to 3X. However, the inoculated strain significantly increased the root N, shoot N, root P, shoot P, SY and SP at all concentration of pesticides compared to uninoculated plants. For example, *Rhizobium* strain MRL3 when used with pesticides, increased the root N, shoot N, root P, shoot P, SY and SP by 30, 7, 41, 21, 55 and 6%, respectively, at 3X of clodinafop and 33, 8, 61, 26 and 111 and 5%, respectively, at 3X of

tebuconazole when compared to the treatments with the same dose of pesticides but devoid of inoculant. The study thus suggested that the pesticide tolerant rhizobial strains (*Mesorhizobium* strain MRC4, *Rhizobium* strain MRP1, *Bradyrhizobium* strain MRM6 and *Rhizobium* strain MRL3) or phosphate solubilizing strain (*Pseudomonas aeruginosa* strain PS1) due to their intrinsic abilities of growth promotion and attenuation of the toxic effects of pesticides could be developed as inoculant and be exploited for remediation or restoration of pesticide polluted soils.

There has been a tremendous research on enhancing crop productivity through the introduction of PGPR in conventional soils applying different methods to find out both the super-inoculant and the strategies as to how the productivity could be improved. The challenge now is however to develop different strategies to identify pesticide resistant PGPR that may work competently and simultaneously in geographically and agronomically different soils. Furthermore, exploration of novel genes expressing greater potential of degradation or transforming a wide range of agrochemicals including herbicides/insecticides/fungicides among the heterogeneous bacterial communities inhabiting the pesticide stressed environment and enhancing the plant growth promoting efficiency of the pesticide resistant plant growth promoting rhizobacteria through genetic manipulation may provide new insights to upgrade economically feasible and ecologically sound agriculture systems.